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Studies on the Bacterial Decomposition in the Meat of Aquatic Animals

MASAO KIMATA

(Received March 31 st, 1941)

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Introduction.

After the death, a living matter is of itself decomposed by the action of enzymes inherent in the tissues. This phenomenon is the autolysis or the autodigestion, as is known by many researches hitherto reported. In general, however, the tissue after death is not only decomposed by autolytic enzymes but also by various microorganisms existing in the natural world, that is to say, the compound phenomenon, the so-called decaying or spoilage, takes place. If enzymes contained in the tissue are destroyed by heating or by other means, the action of microorganisms alone remains as the cause of decaying.

As for the decaying, the studies hitherto reported have taken two courses, in one of which chemical changes and decomposition products, while in the other, microorganisms causing decomposition have mainly drawn attention.

It is believed that the meat of aquatic animals such as fishes, crustacea, etc. is subjected to the decaying much quicker than that of animals living on land and in air, and that even among the aquatic animals the rapidity of decaying differs with the kind of them, viz. certain fish decays more rapidly than the other fish, and, in general, crustacea and mollusca decay more easily than fishes. For instance, sea bream can be kept in an apparently edible condition for considerable time, while both lobsters and clams decompose very rapidly after death.

Notwithstanding the intensive researches, there are rather comparatively scarce studies of decaying from the bacteriological point of view, little being known scientifically. There are, indeed, a few reports of the studies on the distribution of bacteria in the decomposed fish meats and on the change of number of bacteria during decaying processes. But the results of such studies may appear in wide difference in accordance with various conditions. With the view of making clear such circumstances, the present author has made a series of systematic studies; which includes the bacteriological examination of, and the determination of the curve of progress of, bacterial decomposition in the meats of aquatic animals as well as the analysis of the effects upon them of physical and chemical changes in environmental conditions.

The author is of opinion that in any problem of preservation and manufacturing of aquatic products, the important significance of such studies must be taken into consideration.

The present report consists of four parts. In Part One, the author concerns with the studies of the decomposing bacteria of meat of aquatic animals, in Part Two, with the progress of bacterial decomposition of meat of aquatic animals, and the cause of difference in it with the kind of animals, in Part Three, with the relation between autolytic and bacterial agencies in the decomposition of muscles, and lastly in Part Four, with the effects of various physical and chemical environmental changes upon the progress of bacterial decomposition.

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History.

The decomposing bacteria for the meat of aquatic animals vary in species and in characteristics. So-called usual decaying bacteria are principally water bacteria from marine or fresh water origin. Many investigators have reported that those bacteria are present in slime, gill and intestinal canal of living fishes, and immediately grow when the fishes die, causing decomposition. According to HUNTER,⁽⁵⁴⁾ LUMLEY, PIQUÉ and REAY,⁽⁷¹⁾ GROSS,⁽⁴²⁾ and HOUGHTON and HUNTER⁽⁶³⁾ fishes are kept fresh in comparatively long time, if the outside of the body (especially the slime) is thoroughly washed, and the gill and the viscera are carefully removed. (Because the muscle is avoided from contamination caused by the bacteria existing in them). Recently SCHWARTZ⁽⁹⁸⁾ described that when fishes are kept at various temperatures between 6°C and 0°C, if the body-slime is removed by washing, the number of bacteria existing in it decreases, but, in spite of that, the keeping quality is not enlarged, because of remaining bacteria, which very quickly grow and invade into the muscle from the injured part of the skin. This fact is already pointed out by HARRISON and SADLER.⁽⁴⁸⁾

Studying on the bacteria in the body-slime of haddock, STEWART⁽¹⁰⁷⁾ isolated 247 strains from the fishes freshly caught and 137 strains from market fishes which are stored in 7-8 days after death. These microorganisms were found to be as follows, but groups of *Aerobacter*, *Escherichia*, *Proteus* and *Bacillus* were not found.

	Fresh	Market
<i>Achromobacter</i>	57%	64%
<i>Micrococcus</i>	22	11
<i>Flavobacterium</i>	11	20
<i>Serratia</i> }	5	2
<i>Luminous</i> }		
<i>Pseudomonas</i>	5	3

From this fact it is clear that these bacteria grow very quickly after the death of fish. They have also strong decomposing power of protein. So, STEWART presumed that they are the important kinds of the decomposing bacteria for fishes. Moreover, he found that *Flavobacterium* and *Pseudomonas*, isolated from market fish, have strong decomposing power of protein.

According to REED, RICE and SINCLAIR,⁽⁸⁸⁾ when haddock muscle was mixed with the body-slime and the intestinal contents and left standing at 20°C, a large number of *Proteus*-like organisms was found in the beginning, but after the time progresses, *Pseudomonas* grows very rapidly just as the pure culture of it.

GIBBONS⁽³³⁾ isolated 580 strains from the body-slime and the intestinal contents of several marine fishes. These belonged to *Achromobacter*, *Micrococcus*, *Flavobacterium*, *Pseudomonas*, *Bacillus*, *Eberthella* and *Proteus*, among which the first two were predominant in the greater part. The difference between the slime and the intestinal flora was not found.

SANBORN⁽⁹⁶⁾ isolated numerous species belonging to *Achromobacter*, *Pseudomonas*, *Micrococcus*, *Flavobacterium* and *Rhodococcus* from the sea water (from the surface and a depth of 20 meters or of 30-60 fathoms at a quarter of a mile or more from shore) and the slime of several marine fishes. According to him, of these bacteria *Achromobacter pellucidum*, *Pseudomonas fluorescens* and *Rhodococcus agilis* are predominant forms in the proteolytic activity. Also to the list of proteolytic marine species, *Achromobacter geniculatum* and *Flavobacterium fucatum* should be added.

HARRISON⁽⁴⁶⁾ reported nearly the same fact as in GIBBONS' paper. According to him,^(46, 48) however, *Pseudomonas fluorescens* is not of marine but of fresh water origin, and is present in ice used for storage

of fishes. But it is not only verified by BURKE's experiment⁽¹⁹⁾ that marine forms and fresh water forms can survive in sea water as well as fresh water, but also *Pseudomonas fluorescens* is isolated from sea water as described by the above mentioned other investigators.

REED and SPENCE⁽⁸⁷⁾ isolated the flora from the slime and the alimentary tract of numerous haddock as shown in the following table:

	Ali. tra.	Slime
<i>Proteus</i>	70%	18%
<i>Achromobacter</i>	4.4	23
<i>Pseudomonas</i>	8.7	22
<i>Flavobacterium</i>	5.6	8
<i>Coli-Aerogenes</i>	4.6	—
<i>Bacillus</i>	5.7	24
<i>Micrococcus</i>	—	4
Others.....	1	—

Recently SNOW and BEARD⁽¹⁰¹⁾ isolated microorganisms belonging to *Achromobacter*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Kurthia* and *Proteus* from living salmon, stored salmon (at cannery), sea water and water of the estuary. According to him these bacteria play an important rôle on the early stage of the decomposition of salmon, and also *Coli-Aerogenes* groups are found only in the fish caught from the contaminated sea water near the shore.

HARRISON, PERRY and SMITH⁽⁴⁷⁾ studied the spoiling of meat of several marine fishes and found numerous bacteria existing in the slime and the gill. And they believed that *Pseudomonas* and *Achromobacter* are principally decomposing agencies of fish meats.

GOTO⁽³⁹⁾ isolated 63 strains from various decaying fish meat,—viz. *Pseudomonas* 26, *Achromobacter* 28, *Flavobacterium* 3, *Kurthia* 3, *Serratia* 1, *Bacillus* 1, *Micrococcus* 1.

Results obtained from the above mentioned descriptions show that *Pseudomonas*, *Achromobacter*, *Flavobacterium* and *Micrococcus* are always present on the surface and the intestine of fish, and the first three principally cause decaying of fish meat. It is already recognized from past year by many investigators that *Pseudomonas fluorescens* has a very strong antagonistic action against the other microorganisms and various bacteria are inhibited the growth or killed on account of the growth of it. From this point, it is thought that *Pseudomonas fluorescens* play an important rôle on the decomposition of fish meats.

HARRISON and SADLER^(46, 48) ascertained that the yellow or yellowish green discolouration of ice-storage or freezing halibut is produced by the growth of *Pseudomonas fluorescens*.

In early time, BROWNE⁽¹⁶⁾, OBST⁽⁷⁹⁾, HUNTER⁽⁶⁵⁾ and FELLERS^(28, 29) reported that *Coli-Aerogenes* group and spore-forming anaerobes are present in the intestine of fishes and much concerned with the decomposition of fish meats. However, it was afterwards thought that that was so under a special circumstance. Most recently, YASUKAWA⁽¹¹⁹⁾ and TANIKAWA⁽¹⁰⁶⁾ ascertained that *Coli*-type, existing in the alimentary tract of fishes, is of the fish-type which has peculiar characters. From this fact it is thought that the flora previously classified as the *Coli*-group isolated from fish, varies much from that originated from land-living animals and may resemble *Achromobacter*.

GIBBONS⁽³⁴⁾ isolated 34 strains of lactose fermenting bacteria from 112 marine fishes, and he found that 10 strains of them are *Aerobacter* (8 strains are *Aero. aerogenes* and 2 strains are *Aero. cloacae* var.) and 24 strains are *Escherichia*. But, according to him *E. coli*, *E. communior* and *Aero. aerogenes* are not normal intestinal flora of marine fishes but they are present in the intestine of fishes caught from the contaminated sea water near the shore.

It is reported that bacteria are present in the muscle of living fishes and they are in close connection with the decomposition of the dead fish. According to ULRICH⁽¹¹³⁾ there are *Coli* and *Proteus* in the muscle of various fishes. GEE⁽³²⁾ obtained the microorganisms from the muscle of 5 among total 48 of several kinds of fishes. The forms isolated were *B. mesentericus vulgatus*, Gram-negative short rods and *Diplococcus*. *B. mesentericus vulgatus* was pleomorphic, and had variable forms (coccus-like or long rods). Also, its physiological characteristics was due to the condition, but generally it had strong proteolytic activity. Therefore, it may be in close connection with the decomposition of fishes. However, according to many investigators fish muscles are sterile and do not contain the living bacteria. GEE obtained it from small part of all samples of the muscle and also isolated the same organism from the slime of the body. From this point it is thought that his experiment was contaminated with the slime.

Little is known of the decomposing bacteria of meat of crustacea. From its life form, it is presumed that the decomposing bacteria are the same in the case of fishes. According to HARRIS⁽⁴⁵⁾ bacteria in connection with the decomposition of crab meat belong to *Proteus*, *Pseudomonas* and *Flavobacterium*. According to REED and McLEOD⁽⁸⁹⁾ the decomposition of lobster meat is caused by *Pseudomonas*, *Flavobacterium* and *Bacillus* existing in the intestinal canal.

The decomposing bacteria of shell-fish meat is nearly the same

as in the case of fishes and crustacea. But in this case, it is thought that *Coli*-group is somehow connected with the decomposition, seeing from their habitat. SYMONS⁽¹⁰⁵⁾ found that there is *Pseudomonas fluorescens* in the clam (*Mya arenaria*). RICE⁽⁹²⁾ isolated 14 *Bacillus*, 6 *Pseudomonas*, 3 *Proteus*, 3 *Flavobacterium*, 3 *Achromobacter*, 3 *Aerobacter* and 3 *Escherichia*, and ascertained that they have relation to the decomposition. HUNTER and LINDEN⁽⁸⁸⁾ isolated *Serratia*, *Pseudomonas*, *Proteus*, *Clostridium*, *Bacillus*, *Aerobacter* and *Escherichia* as the decomposing bacteria of oysters (after becoming acidic in condition such as pH=4.6-5.0, with progress of the decomposition, *Streptococcus*, *Lactobacillus* and yeast appear). ELIOT⁽²⁶⁾ isolated *Coli*-group, *Streptococcus*, *Pseudomonas*, pigment formed coccus and aerobic spore forming rods and found that the most of the formers are in close connection with the decomposition while the latter ones are accessory. And then BACON⁽⁷⁾ ascertained ELIOT's experiment. AKIBA⁽¹⁾ isolated *Coli*-group, *Coli*-like forms, non-pigment forming rods (water bacteria), *Vibrios*, pigment forming rods, spore forming rods and *Streptococcus* from decaying oysters and found that the most of them belong to *Coli*-group. More recently TANIKAWA⁽¹⁰⁷⁾, in the bacteriological studies of low temperature storage of oysters, has isolated *Achromobacter*, *Flavobacterium*, *Pseudomonas* and *Micrococcus*, which cause decomposition. And also, in the biological studies on the organisms of *Coli*-group isolated from the intestinal canal of oysters, he⁽¹⁰⁶⁾ ascertained that only feces forms of the fish-type are isolated from oysters, which have inhabited the place, far away from shore and uncontaminated by sewage from land.

Eventually, it appears that the decomposing bacteria of shell-fish caught in uncontaminated place are nearly the same as those of fishes but in contaminated place *Coli*-group is added.

All of the above mentioned articles are the studies on the decomposing bacteria of meat of aquatic animals. Studies on the progress of the bacterial decomposition of meat of aquatic as well as land animals, so far as the author is aware, are rather few, only a little having been published on it. From the studies of autolysis and bacterial decomposition of haddock, lobster and clam muscles, REED, RICE and SINCLAIR⁽⁸⁸⁾ concluded that autolysis alone and autolytic and bacterial decomposition are nearly identical for the early stage (the first 8-24 hours), and that the difference of decomposition of different animal tissues is the results of difference in autolysis rather than of differences in the bacterial action.

PART ONE

Preliminary Experiment

(Decomposing Bacteria for the Meat of Aquatic Animals)

From the above mentioned articles it appears that the bacterial decomposition of meat of aquatic animals takes place by the action of water bacteria belonging to *Pseudomonas*, *Achromobacter*, *Flavobacterium* and *Micrococcus*. However, only a few papers have been reported with respect to the relation between the decomposing action and the kind of microorganisms.

Part One contains the confirmation of the above mentioned facts and the results of preliminary experiments for determining microorganisms which must be employed in the studies described under Part Two.

I. Isolation of Bacteria from Decomposing Materials.

Method: The following kinds of aquatic animals were obtained from the fish market from time to time throughout a year, namely, tunny, *Thunnus orientalis* (TEMMINCK and SCHLEGEL); bonito, *Katsuwonus vagans* (LESSON); chub mackerel, *Scomber japonicus* (HOUTTYUN); salmon, *Oncorhynchus keta* (WALBAUM); carangoid, *Trachurus japonicus* (TEMMINCK and SCHLEGEL); halibut, *Palatichthys olivaceus* (TEMMINCK and SCHLEGEL); sea bream, *Pagrosomus major* (TEMMINCK and SCHLEGEL); carp, *Cyprinus carpio* LINNE; serranid, *Lateolabrax japonicus* (CUVIER); spiny lobster, *Panulirus japonicus* v. SIEBOLD; squid, *Loligo bleekeri* KEFERSTEIN and oyster, *Ostrea gigas* THUNBERG. A bit of each flesh was placed in a Petri's dish at room temperature. Generally, a very unpleasant odour was emitted after 2 or 3 days' storage. The surface of the flesh was covered by a faint white film and the bottom side of the flesh discharged some quantity of mucous. Then the isolation was carried out as usual from the surface and the mucous using the agar plate culture.

Morphological, cultural and physiological characteristics of every one of the isolated microorganisms were studied by the usual method.

Results: Organisms isolated were 3 species, 52 strains of *Pseudomonas*; 12 species, 32 strains of *Achromobacter*; 2 species, 4 strains of *Flavobacterium*; 1 species, 3 strains of *Serratia*; 1 species, 2 strains of

Kurthia; 1 strain of *Bacillus* and 2 species, 3 strains of *Micrococcus*, viz. sum of them is 22 species, 97 strains.

The description and classification that are described according to BERGEY's manual⁽¹⁾ are as follows:

1. <i>Pseudomonas fluorescens</i> (FLÜGGE) MIGULA	27 strains.
2. <i>Pseudomonas ovalis</i> (RAVENEL) CHESTER	9 "
3. <i>Pseudomonas schuylkilliensis</i> (WRIGHT) CHESTER	16 "
4. <i>Achromobacter multistriatum</i> (WRIGHT) BERGEY et al.	8 "
5. <i>Achromobacter sinuosum</i> (WRIGHT) BERGEY et al.	5 "
6. <i>Achromobacter litorale</i> (RUSSELL) BERGEY et al.	2 "
7. <i>Achromobacter ambiguum</i> (WRIGHT) BERGEY et al.	2 "
8. <i>Achromobacter inunctum</i> (POHL) BERGEY et al.	1 strain.
9. <i>Flavobacterium turcosum</i> (ZIMMERMANN) BERGEY et al.	3 strains.
10. <i>Flavobacterium lutescens</i> (MIGULA) BERGEY et al.	1 strain.
11. <i>Serratia marcescens</i> BIZIO	3 strains.
12. <i>Kurthia zopfii</i> (KURTH) TREVISAN	2 "
13. <i>Bacillus subtilis</i> (EHRENBERG) COHN	1 strain.
14. <i>Micrococcus candidus</i> COHN	1 "

As can not be identified by the already published literatures, the following species seem to be new species.

15. <i>Achromobacter concretificum</i> nov. sp.	1 strain.
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Morphology.

Rods: 0.7-0.8 by 1.2-2.5 microns, with rounded ends, occurring singly and in pairs. Motile, possessing one to three polar flagella. It forms no endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: irregular, spreading (effuse), greenish gray, smooth, lobate, grumose, translucent, finely or coarsely granular.

Agar slant: gray, spreading, effuse, glistening, translucent.

Gelatin colonies: circular, flat, entire or undulate, amorphous or granular.

Gelatin stab: filiform growth along the line of puncture, no liquefaction.

Potato: thick, moist, glistening, cream yellow.

Broth: turbid with gray sediment.

Physiology.

Litmus milk: acid, coagulated.

Indol is not formed.

Nitrates are reduced to nitrites.

Acid is formed in dextrose, sucrose and lactose.

Starch is not hydrolyzed.

Aerobic, facultative.

Optimum temperature: 25 to 30°C.

This bacterium possesses all the characteristics of the genus *Achromobacter* as given by BERGEY, and although allied to *Achromo-*

bacter ambiguum (WRIGHT) BERGEY et al. and *Achromobacter nijibetsui* TAKEDA (Zentbl. Bakt. 2 Abt., 94, 1936, S. 48), it differs from the former in the arrangement of flagella, reduction of nitrates, formation of indol and optimum temperature, and differs from the latter in its action on milk and in many respects of the cultural characteristics. As it does not appear to have been described previously, the name *Achromobacter concretificum* is proposed.

16. *Achromobacter sordidum* nov. sp. 1 strain.

Morphology.

Rods: 0.7-0.8 by 1.2-2.5 microns, with rounded ends, occurring singly. Motile, possessing single polar flagellum. It forms no endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: circular, smooth, flat or raised, brownish gray, entire, finely granular. Medium assumes slightly brownish.

Agar slant: filiform, flat, glistening, smooth, translucent, dark gray. Medium assumes slightly brownish.

Gelatin colonies: circular, flat or concave, entire, finely granular, crateriform liquefaction.

Gelatin stab: crateriform liquefaction, filiform growth along the line of puncture.

Potato: thick, moist, glistening, brilliant reddish orange.

Broth: turbid with thin pellicle and sediment.

Physiology.

Litmus milk: becoming alkaline, peptonized. Litmus is reduced very quickly.

Indol is not formed.

Nitrates are reduced to nitrites.

Acid is formed in dextrose but not in sucrose and lactose.

Starch is not hydrolyzed.

Aerobic, facultative.

Optimum temperature: 25 to 30°C.

It possesses all the characteristics of the genus *Achromobacter* as given by BERGEY, and although closely allied to *Achromobacter putrefaciens* DERBY and HAMMER, it differs in its action on carbohydrates and in a few respects of the cultural characteristics. As it does not appear to have been described previously, the name *Achromobacter sordidum* is proposed.

17. *Achromobacter tabificum* nov. sp. 4 strains.

Morphology.

Rods: 0.5-0.7 by 0.7-2.0 microns, short rods, with rounded ends, occurring singly and in pairs. Motile, possessing polar flagellum. It forms no endospore. It readily stains with all common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: irregular, raised, smooth, opaque, gray, auriculate, finely or coarsely granular.

Agar slant: spreading, smooth, moist, raised, glistening, gray. Medium assumes slightly brownish.

Gelatin colonies: circular, translucent, entire, granular, crateriform liquefaction.
 Gelatin stab: rapid liquefaction, stratiform or infundibuliform.
 Potato: cream yellow, moist, spreading, glistening. Medium assumes slightly gray.
 Broth: strongly turbid with slight pellicle and flocculent sediment.

Physiology.

Litmus milk: acid reaction, soft coagulum, slow peptonization.
 Indol is not formed.
 Nitrates are reduced to nitrites.
 Acid is formed in dextrose and sucrose, but not in lactose.
 Starch is not hydrolyzed.
 Aerobic, facultative.
 Optimum temperature: 25 to 30°C.

It possesses all the characteristics of the genus *Achromobacter*. But as it appears that there are no bacteria which are analogous to this bacterium, the name *Achromobacter tabificum* is proposed.

18. *Achromobacter piscarium* nov. sp. 4 strains.

Morphology.

Rods: 0.6-0.7 by 1.0-2.5 microns, with rounded ends, occurring singly and in pairs. Motile, possessing one to four polar flagella. It forms no endospore. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: circular or amoeboid, whitish, flat or raised, smooth, entire, translucent to opaque, coarsely or finely granular.
 Agar slant: filiform, raised, smooth, glistening, translucent.
 Gelatin colonies: spot, flat, entire, gray, finely granular, no liquefaction.
 Gelatin stab: villous growth along the line of puncture, liquefaction saccate (very slowly liquefied).

Potato: moist, spreading, yellowish gray.
 Broth: turbid with thin pellicle and gray sediment.

Physiology.

Litmus milk: becoming acidic slowly.
 Indol is not formed.
 Nitrates are reduced to nitrites.
 Acid and gas are formed in dextrose and sucrose.
 Starch is not hydrolyzed.
 Aerobic, facultative.
 Optimum temperature: 25°C.

This bacterium resembles *Achromobacter tabificum* nov. sp., but it differs in its action on gelatine, in the arrangement of flagella and in many respects of cultural features. Therefore, the name *Achromobacter piscarium* is proposed.

19. *Achromobacter gotoi* nov. sp. 1 strain.

Morphology.

Rods: 0.6-0.7 by 1.0-2.0 microns, with rounded ends, occurring singly and in short chains. Motile, possessing one to three polar flagella. It forms no endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: irregular, spreading, translucent, smooth, flat or effuse, auriculate. finely granular.

Agar slant: spreading, translucent, smooth, flat or effuse, glistening.

Gelatin colonies: small circular, smooth, entire, gray.

Gelatin stab: villous growth along the line of puncture, no liquefaction.

Potato: thin, moist, pinkish gray to dirty orange.

Broth: turbid with reticulose pellicle and sediment.

Physiology.

Litmus milk: alkaline.

Indol is not formed.

Nitrates are reduced to nitrites (weak).

Acid is formed in dextrose (weak).

Starch is not hydrolyzed.

Aerobic, facultative.

Optimum temperature: 25 to 30°C.

It is different from any one of genus *Achromobacter* which has polar flagella and does not liquefy gelatine. But, as it appears to have been closely allied to Goto's *Achromobacter* F (Bull. Jap. Soc. Sci. Fish., 2, 1933, p. 59), the name *Achromobacter gotoi* is proposed.

20. *Achromobacter hukagawae* nov. sp.

1 strain.

Morphology.

Rods: 0.6-0.8 by 1.0-2.5 microns, with rounded ends, occurring singly, in pairs and in short chains. Motile, possessing polar flagella. It forms no endospore. It readily stains with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: circular, smooth, raised or convex, gray, entire, granular

Agar slant: filiform, raised, smooth, glistening, translucent, gray.

Gelatin colonies: small circular, smooth, flat or raised, entire, amorphous or finely granular.

Gelatin stab: filiform growth along the line of puncture, no liquefaction.

Potato: thick, moist, glistening, dark orange or yellowish brown.

Broth: turbid with thin pellicle and slight sediment.

Physiology.

Litmus milk: very weak acid or unchanged.

Indol is not formed.

Nitrates are not reduced.

Acid is formed in dextrose, but not in sucrose and lactose.

Starch is not hydrolyzed.

Aerobic, facultative

Optimum temperature: 25°C.

It resembles *Achromobacter sinuosum* (WRIGHT) BERGEY et al. But it differs in the production of indol and in several cultural features. It is recognized as a new species, and is named *Achromobacter hukagawae*.

21. *Achromobacter imbecillum* nov. sp.

2 strains.

Morphology.

Rods: 0.6-0.7 by 1.2-2.0 microns, with rounded ends, occurring singly, in pairs

and in short chains. Non-motile. It does not form endospores. It stains readily with all the common stains, but is Gram-negative.

Cultural characteristics.

Agar colonies: circular, smooth, convex, gray, translucent, finely granular.
 Agar slant: filiform or echinulate, smooth, raised, gray, opaque, glistening.
 Gelatin colonies: small circular, smooth, raised or convex, gray.
 Gelatin stab: filiform growth along the line of puncture, no liquefaction.
 Potato: thick, glistening, orange yellow. Medium assumes dark gray.
 Broth: turbid with thin pellicle and slight sediment.

Physiology.

Litmus milk: unchanged.
 Indol is not formed.
 Nitrates are not reduced.
 Acid is not formed in carbohydrates media.
 Aerobic, facultative.
 Optimum temperature: 25 to 30°C.

As it appears that this bacterium is quite different from any one of genus *Achromobacter* which is non-motile and does not liquefy gelatine, the name *Achromobacter imbecillum* is proposed.

22. *Micrococcus purus* nov. sp.

2 strains.

Morphology.

Spheres: 0.7-1.0 microns, occurring singly, in pairs and in irregular clumps.
 Non-motile. Gram-positive.

Cultural characteristics.

Agar colonies: circular, white, glistening, convex or pulvinate, smooth, entire, amorphous or finely granular.
 Agar slant: filiform, white, raised or convex, glistening, smooth, opaque.
 Gelatin colonies: circular, smooth, white, entire.
 Gelatin stab: saccate or infundibuliform liquefaction, with heavy white sediment.
 Potato: thin, white streak, glistening.
 Broth: turbid with little white sediment. No pellicle.

Physiology.

Litmus milk: acid.
 Indol is not formed.
 Nitrates are reduced to nitrites.
 Acid is formed in dextrose and sucrose, but not in lactose.
 Starch is not hydrolyzed.
 Aerobic, facultative.
 Optimum temperature: 25 to 30°C.

It possesses all the characteristics of the genus *Micrococcus* as given by BERGEY, although it resembles *Staphylococcus albus* ROSENBAACH in many respects. But it is quite different from any one of genus *Micrococcus* which does not produce chromogenesis and liquefies gelatine. And it closely allied to *Micrococcus solidus* GOTO and KIMATA (Zentbl. Bakt. 2 Abt., 90, 1934, S. 148). It differs, however, in size of cells and in several respects of cultural features. As it does not

appear to have been described previously, the name *Micrococcus purus* is proposed.

As mentioned above, the majority of the organisms are water bacteria which are gram negative, slender, active motile short rods. Indol is not formed. Optimum temperature lies between 25°C and 30°C. The organisms belonging to *Pseudomonas*, are little in number of species but great in number of strains. On the contrary, *Achromobacter* is great in number of species, but few in strains. From this fact, it is thought that the decomposition of aquatic animals is due to the water bacteria and especially *Pseudomonas* play an important part in it.

II. Decomposing Action of the Isolated Bacteria.

Experiment 1.

To determine whether the above isolated bacteria grow well on fish meat and produce unpleasant odour or not, a piece of tunny and serranid placed into Petri's dish aseptically was inoculated with each of the isolated bacteria. Every one of these grew well on the fish meat and produced unpleasant odour though in different degrees. The predominating organisms were *Pseudomonas fluorescens*, *Achromobacter tabificum*, *Pseudomonas schuylkilliensis* and *Serratia marcescens*.

Obtaining of the fish meat aseptically is very difficult in comparison with that of land animals and in many cases it is contaminated with the flora on the outside of the body. Therefore, in this experiment the following method was used.

The fish body was washed with sterilized water, wiped off the slime, disinfected with alcohol and corrosive sublimate solution, and stripped off the skin with a sterilized knife and then cut off the muscle with other sterilized knife. A piece of the muscle was immersed in alcohol for several seconds, taken out from alcohol and burnt off the alcohol by a flame and then cut off coagulated part of outside with a sterilized knife again. Non-coagulated fresh muscle of the interior was placed in a sterilized Petri's dish and afterwards sterile state by incubating for 24 hours was ascertained, and then inoculated with the bacteria. According to this method fish meat may be obtained aseptically, without contaminations.

Experiment 2.

Present experiment embodies the author's observations on the relation between the decomposition of fish meat and kinds of bacteria.

Muscle suspension was made and inoculated with pure or mixed cultures, and the number of living cells, quantity of volatile basic nitrogen and formol titrating nitrogen were measured at definite intervals.

Muscle suspension: Five hundred grams of fish muscle (chub mackerel) were added to 2,000 c.c. of distilled water, heated in Koch's steam sterilizer for 2 hours, finely granulated and then was filled to 3,000 c.c. with distilled water.

Organisms employed: *Pseudomonas fluorescens*, *Pseudomonas schuyllkilliensis*, *Pseudomonas ovalis*, *Achromobacter multistriatum*, *Achromobacter tabificum*, *Serratia marcescens*, *Micrococcus purus*, *Proteus vulgaris* and *Staphylococcus aureus*. The last two were got from the Imperial Institute for Infectious Diseases and the others were previously isolated from decomposing materials.

Procedure: One hundred and fifty cubic centimeters of the muscle suspension were put into sterilized flasks of a capacity of 500 c.c. and added with an equal volume of distilled water and sterilized. Broth cultures of various kinds of bacteria (at 28°C for 48 hours) were diluted to 10,000 times with sterilized distilled water. To the above flasks 3 c.c. of these bacterial suspensions were added and were incubated at 27–28°C. At definite intervals, 20 c.c. of the mixtures were taken out, and volatile basic nitrogen (in 10 c.c.) and formol titrating nitrogen (in 5 c.c.) were measured by SÖRENSEN'S method. Bacterial counts in 1 c.c. were determined with agar plate culture by means of the dilution method.

Results: Results obtained are shown in Tables 1 and 2, and also in Fig. 1, representing the data in Table 1. From these results it is clear that the production of volatile basic nitrogen and that of formol titrating nitrogen run nearly parallel to each other during decomposition due to the action of the same organism. The production of these nitrogens much differs according to the kinds of organisms employed. In the case of the mixed culture, with *Pseudomonas fluorescens*, *Achromobacter multistriatum*, *Proteus vulgaris* and *Staphylococcus aureus*, after 3 days it appeared approximately just as the pure culture of *Pseudomonas fluorescens*, and the degree of the decomposition seems to be nearly the same as that in the pure culture of *Pseudomonas fluorescens*. Within the limits of this experiment the degree of the decomposition is greater by the water bacteria than by the others.

In logarithmic growth phase, the growth of bacteria does not much differ among the water bacteria such as *Pseudomonas*, *Achromobacter* and *Serratia*, but the growth rates of *Staphylococcus aureus*

Table 1. Production of formol titrating nitrogen and volatile basic nitrogen in bacterial decomposition of fish muscle suspension.

Time of incubation	<i>Pseudomonas fluorescens</i>				<i>Achromobacter multistriatum</i>			
	Formol titr. N		Volatile bas. N		Formol titr. N		Volatile bas. N	
	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	0.420	—	0.315	—	0.420	—	0.315	—
1	0.420	0	0.350	0.035	—	—	0.350	0.035
3	0.613	0.193	0.835	0.520	0.490	0.070	0.770	0.455
5	1.265	0.840	1.838	1.523	0.805	0.385	1.470	1.155
7	1.593	1.173	2.730	2.415	0.980	0.560	1.960	1.645
9	1.785	1.365	3.255	2.940	1.103	0.683	2.205	1.890
12	1.960	1.540	3.990	3.675	1.120	0.700	2.205	1.890
15	1.960	1.540	3.990	3.675	1.120	0.700	2.205	1.890

Table 1. continued.

Time of incubation	<i>Serratia marcescens</i>				<i>Staphylococcus aureus</i>			
	Formol titr. N		Volatile bas. N		Formol titr. N		Volatile bas. N	
	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	0.420	—	0.315	—	0.420	—	0.315	—
1	—	—	0.403	0.088	0.420	0	0.315	0
3	0.770	0.350	0.875	0.560	0.420	0	0.455	0.140
5	1.855	1.435	3.255	2.940	0.525	0.105	0.508	0.193
7	3.215	2.795	5.250	4.935	0.595	0.175	0.508	0.193
9	3.605	3.185	7.035	6.720	0.718	0.298	0.578	0.263
12	4.743	4.323	9.310	8.995	0.858	0.438	0.823	0.508
15	4.743	4.323	9.345	9.030	1.155	0.735	1.400	1.085
20	—	—	—	—	2.105	1.685	3.010	2.695

Table 1. continued.

Time of incubation	<i>Proteus vulgaris</i>				<i>Achromobacter tabificum</i>			
	Formol titr. N		Volatile bas. N		Formol titr. N		Volatile bas. N	
	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	0.420	—	0.315	—	0.508	—	0.315	—
1	0.420	0	0.350	0.035	—	—	—	—
3	0.438	0.018	0.455	0.140	—	—	—	—
5	0.438	0.018	0.560	0.245	1.523	1.015	2.275	1.960
7	0.438	0.018	0.683	0.368	2.066	1.558	3.080	2.765
9	0.630	0.210	0.875	0.560	—	—	—	—
12	0.770	0.350	1.295	0.980	—	—	—	—
15	0.840	0.420	1.400	1.085	—	—	—	—
20	0.893	0.473	1.435	1.120	2.275	1.767	3.693	3.378

Table 1. continued.

Time of incubation	<i>Pseudomonas ovalis</i>				<i>Pseudomonas schuylkilliensis</i>			
	Formol titr. N		Volatile bas. N		Formol titr. N		Volatile bas. N	
	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	0.508	—	0.315	—	0.508	—	0.315	—
5	1.120	0.612	1.470	1.155	2.135	1.627	2.870	2.555
7	1.330	0.822	2.293	1.978	2.730	2.222	4.113	3.798
20	1.383	0.875	2.800	2.485	3.395	2.887	5.740	5.425

Table 1. continued.

Time of incubation	Mixed culture of <i>Ps. fluorescens</i> , <i>Achr. multistriatum</i> , <i>Prot. vulgaris</i> and <i>Staph. aureus</i>				<i>Micrococcus purus</i>			
	Formol titr. N		Volatile bas. N		Formol titr. N		Volatile bas. N	
	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	0.420	—	0.315	—	0.508	—	0.315	—
1	0.420	0	0.368	0.053	—	—	—	—
2	0.455	0.035	0.490	0.175	—	—	—	—
4	0.945	0.525	1.050	0.735	—	—	—	—
5	—	—	—	—	0.508	0	0.455	0.140
7	1.610	1.190	2.765	2.450	0.508	0	—	—
9	1.820	1.400	3.255	2.940	—	—	—	—
12	1.995	1.575	3.990	3.675	—	—	—	—
15	2.030	1.610	4.025	3.710	—	—	—	—
20	—	—	—	—	0.840	0.332	1.120	0.805

Table 2. Growth of bacteria.

Microorganisms	Time of incubation					
	0	21 h.	2 d.	3 d.	4 d.	5 d.
<i>Pseudomonas fluorescens</i>	886	536×10^5	137×10^7	138×10^7	427×10^6	580×10^6
<i>Achromobacter multistriatum</i>	229	341×10^5	644×10^6	133×10^7	162×10^7	680×10^6
<i>Serratia marcescens</i>	783	324×10^5	713×10^6	175×10^7	138×10^7	183×10^7
<i>Staphylococcus aureus</i>	473	483×10^4	178×10^6	451×10^6	211×10^6	171×10^6
<i>Proteus vulgaris</i>	226	317×10^3	111×10^6	135×10^6	328×10^6	385×10^6
<i>Pseudomonas ovalis</i>	487	711×10^5	—	—	—	—
<i>Pseudomonas schuylkilliensis</i>	598	410×10^5	—	—	—	—
<i>Achromobacter tabificum</i>	689	763×10^5	—	—	—	—
<i>Micrococcus purus</i>	265	128×10^2	—	—	—	—

Micrococcus purus and *Proteus vulgaris* are smaller than that of the formers.

From the above mentioned facts, it is clear that the decomposing action and the multiplication of microorganisms on fish muscle must

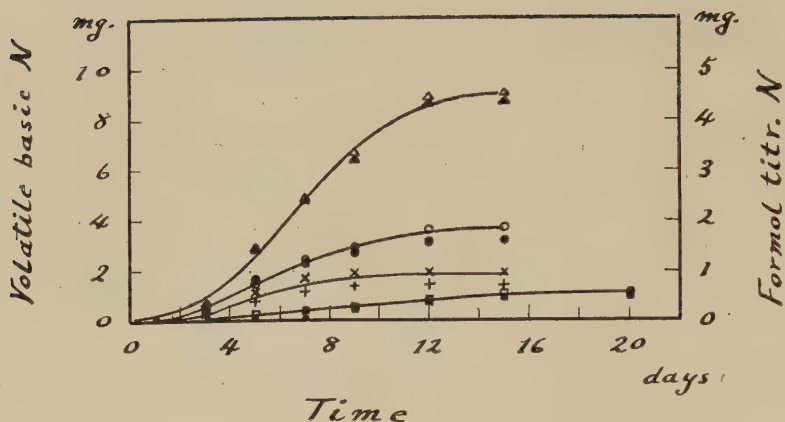


Fig. 1. Production of formol titrating nitrogen and volatile basic nitrogen in bacterial decomposition of fish muscle suspension.

- | | | |
|--------|--------------------|--|
| ○..... | volatile basic N | } <i>Pseudomonas fluorescens</i> . |
| ●..... | formol titrating N | |
| △..... | volatile basic N | } <i>Serratia marcescens</i> . |
| ▲..... | formol titrating N | |
| ×..... | volatile basic N | } <i>Achromobacter multistriatum</i> . |
| ÷..... | formol titrating N | |
| □..... | volatile basic N | } <i>Proteus vulgaris</i> . |
| ■..... | formol titrating N | |

be strictly distinguished. Namely, even when various organisms have the same rate of their multiplication, the decomposing action of them may very much vary from each other.

The limit of growth is reached by any kinds of the bacteria in about 2 days, and after the living cells attain the greatest number the progress of the decomposition becomes suddenly faster.

III. Summary.

The bacterial decomposition of meat of aquatic animals seems to be due to the water bacteria such as *Pseudomonas*, *Achromobacter* and *Flavobacterium*, and the decomposing action of these organisms may be stronger than that of *Proteus vulgaris* and *Staphylococcus aureus*.

The decomposition of the muscle is suddenly progressed when the organisms in muscle grow to about the greatest number.

The degree of the decomposing action and the rate of the multiplication of organisms in fish muscle are not always in agreement with each other.

PART TWO

Bacterial Decomposition of Meat of Aquatic Animals.

It is generally accepted that the decomposition of meat of aquatic animals is much quicker than that of land animals, and also that the rapidity of the decomposition is different with kinds of animals. For example the mackerel is believed to decompose more easily than the sea bream. Scientific studies on these problems, however, are rather few.

The present study aims to make sure of these facts and to ascertain the cause for these to occur.

I. Experimental Method.

1. *Preparation of samples.*

If a piece of muscle were used for studying the decomposition of meat, as the sample, it would closely resemble the natural condition, a condition that affords the best means. But in order to compare the results obtained among many samples, many pieces of muscle must be prepared with equal volume and equal area, for difference in volume and surface area of the sample do affect the degree of the bacterial growth on them. On the other hand, in order to investigate the change accompanied at successive intervals, just the corresponding part must be taken out from all of the samples. If the part lies near the surface, the number of bacteria is large, but if it is interior, the number is small. Again, the results obtained may be different according to the nature of the surface, whether rough or smooth. These questions are raised not only from the point of view of the bacterial growth but also of the chemical changes. It is difficult to take just the corresponding part out from different pieces of the semi-solid material such as fish meat. When the studies are to be done on many samples, therefore, the use of a piece of muscle as a sample complicates the method and liable makes the results obtained erroneous. If the sample in liquid state is used in such a case, the method may be comparatively simplified and admit less errors. For this reason, the present author employed the samples prepared by the following method:

Muscle suspension:—The meat as fresh as possible was granulated and then the connective tissue was removed from it through a strainer. Five hundred grams of the flesh paste were mixed with

2,000 c.c. of distilled water, heated in Koch's steam sterilizer for 1 hour, and thus coagulated meat was finely granulated again and then was filled to 3,000 c.c. with distilled water. This was used as a original (fundamental) muscle suspension. The reason for the heating is to destroy various enzymes in tissues and thus to avert the effect of autolysis.

Solution of extractive matter of meat:—The meat as fresh as possible, was finely granulated, added to twice the weight of physiological salt solution, and kept at about 0°C for 24 hours, and then filtered. The filtrate was heated in Koch's steam sterilizer for 1 hour, and filtered again. This solution was used as a original extractive matter solution.

If the extractive matter is soaked out from the meat by means of heating, it is mixed with gelatine which is made from the connective tissue (collagen) in muscle. But when it is soaked out at low temperature, it is free from gelatine.

Suspension of muscle protein:—Fresh meat was finely granulated. Two hundred and fifty grams of this meat paste were added to 1,500 c.c. of distilled water, heated in Koch's steam sterilizer for 1 hour and filtered. The residue was washed with hot distilled water in several times to remove the soluble matter as enough as possible, and finely granulated, and then filled to 1,500 c.c. with distilled water.

2. *Micoorganisms employed.*

Pseudomonas fluorescens principally was used from the reason of the above mentioned facts. But if necessary, other organisms often were used.

Organisms employed were cultured in broth at 26–27°C for 48 hours. The broth cultures were diluted to 10,000 times with sterilized distilled water and were used. These bacterial suspensions contained about 7×10^4 of living cells in 1 c.c.

3. *Method for the quantitative determination of chemicals.*

For studying the progress of chemical changes in certain material, there are, in general, two methods to estimate the degree of decomposition, namely whether by the decrease of the material or by the increase of the decomposition products. Also, in the case of the bacterial decomposition of the meat, these two ways are used. However, method for the quantitative determination of protein is very complex and liable to produce various errors. Especially, it is very difficult to carry out many exact experiments using small quantity of samples at the same time. Although the bacterial protein

is increased and the muscle protein is decreased with the growth of bacteria, the correct determination of the relation between them is almost impossible. And, moreover, changes of the extractive matter can not be recognized by this method.

Thus, the degree of the bacterial decomposition of meat must be estimated by determining the increase of the decomposition products. Decomposition products which are adequate as the standard for the determination of the degree of the decomposition, are as follows:—non-protein nitrogen, soluble nitrogen, amino nitrogen, ammonium nitrogen, volatile basic nitrogen, hydrogen sulphide, amines, etc. Method for the quantitative determination of these materials is comparatively simple but invariably leads to considerably exact results. It seems to be fit for the experiments dealing with many samples. The measurement of non-protein nitrogen or soluble nitrogen is suitable for the estimation of the degree of decomposition of muscle protein, although it is not suitable for the extractive matter. The meat extract is widely used as the media for the bacterial culture and it is already well known that amino nitrogen and volatile basic nitrogen such as ammonia are produced from it with the growth of bacteria. According to KIMURA and KUMAKURA,⁽⁶⁵⁾ GLASSMAN and ROCHWARGER,⁽³⁷⁾ LÜCK and GERDEL,⁽⁷⁰⁾ TILLMANS and OTTO,⁽¹¹¹⁾ YAMAMURA⁽¹¹⁸⁾ and TANIKAWA,⁽¹⁰⁸⁾ the measuring of volatile basic nitrogen, amino nitrogen or ammonium nitrogen is the best method for the examination of freshness of fishes.

From the consideration already mentioned, the present author holds the opinion that the measuring of volatile basic nitrogen, ammonium nitrogen or amino nitrogen is the best method for the determination of the degree of bacterial decomposition of any of meat, extractive matter or muscle protein, and adopted, therefore, this method chiefly in the present study.

Methods used for the determination of various chemicals were as follows:

Total nitrogen—measured by KJELDAHL's method.

Soluble nitrogen—the equal volume of 10 % trichlor-acetic acid was added to the sample and filtered after protein was precipitated. A definite volume of the filtrate free from protein was taken out, and measured by KJELDAHL's method.

Amino nitrogen—the equal volume of 10 % trichlor-acetic acid was added to the sample and filtered after protein was precipitated. A definite volume was taken out and measured by SÖRENSEN's method.

method or VAN SLYKE'S method.

Volatile basic nitrogen—the sample was made weak alkaline by adding magnesia and distilled into the solution of N/20 H_2SO_4 containing methyl red as an indicator and titrated with the solution of N/40 NaOH. Production of bubbles during the distillation was prevented by adding amyl alcohol or capric alcohol.

Hydrogen ion concentration—measured either colorimetrically using CLARK and LUBS' indicator or electrometrically with a potentiometer.

4. *Method for the determination of the number of living bacterial cells.*

A definite volume of the sample was added to a definite volume of sterilized physiological salt solution and after the suspension was uniformly diluted, a definite volume of it was added to another definite volume of sterilized physiological salt solution, and moreover, diluted by the same way repeatedly in order. And then it was cultured by the agar plate culture method at 26°C – 27°C for 2–3 days, and the number of colonies developed on the plate was counted. The number of living cells in the sample was calculated from this result.

5. *Cultural media.*

Common broth and agar-agar media were used for the culture of bacteria.

6. *Procedure.*

A definite volume of either one of the muscle suspension, the solution of extractive matter or the suspension of muscle protein was put into a sterilized flask of a definite capacity of volume and was filled to a definite volume with distilled water or other materials if necessary and then sterilized. (When the added material is not fit for heating, it is sterilized by means of filtration through Chamberland cylinder, and added to the muscle suspension, etc. already sterilized.). To the flask a definite volume of the above mentioned bacterial suspension was added and was incubated at 26°C – 27°C . After standing, a definite volume of the mixture was taken out at definite intervals, and the number of living cells and chemical materials were determined.

II. Decomposition of Fish Muscle and Rate of Growth of Bacteria in Relation to the Number of Organisms Introduced in the Inoculum.

Many bacteria which are present in slime, gill and intestinal canal of fishes while living, immediately grow when dead, and become the cause of the decomposition of them. According to HUNTER,⁽⁶⁴⁾ LUMLEY, PIQUÉ and REAY,⁽⁷¹⁾ GROSS,⁽⁴²⁾ and HOUGHTON and HUNTER,⁽⁶³⁾ fishes are kept fresh for a comparatively long time, if the slime of the body is sufficiently washed, and gill and viscera are carefully removed (as the muscle is averted from the contamination due to the bacteria present in them). Recently, SCHWARTZ⁽⁹⁸⁾ described that in storage of fishes, when the body-slime is removed by the washing, the number of bacteria which are therein present decreases, but the keeping quality does not increase. The reason is, according to him, that the remaining bacteria very quickly grow and invade into the muscle from injured parts which are made on the skin by the washing. This fact is already pointed out by HARRISON and SADLER.⁽⁴⁸⁾

It is well known that when bacteria are cultured in broth under a certain definite condition, generally the number of living cells reaches a constant irrespective of number of organisms introduced in the inoculum. This is also ascertained in the present author's experiments on the fish muscle suspension, previously described. In the said experiments it was shown that the decomposition of muscle is quickened from the time when the number of living cells reached the constant.

Consequently, if the time required for reaching the maximum growth of bacteria is affected by the initial number of inoculated organisms, the progress of the decomposition of muscle must be different with the number of bacterial cells initially existed in it. So far, however, our knowledge of the relationship between the number of inoculated organisms and the growth rate in the stage preceding to its asymptotic condition is rather scanty, only a few papers having been published on it. RAHN⁽⁸⁶⁾ concluded that the growth rate of bacteria, viz. the generation time is independent of the initial number of organisms introduced in the inoculum. On the other hand, TOMINAGA⁽¹¹²⁾ had studied *Bact. coli* and found that duration of the growth period is independent of the amount of inoculum, namely the generation time depends on the initial number of organisms introduced in the inoculum. Also, MCKENDRICK and PAI⁽⁷⁴⁾ found that duration of the growth

period to reach the maximum is nearly the constant independently of the amount of inoculum. According to RAHN,⁽⁸⁵⁾ SLATOR,⁽¹⁰⁰⁾ PENFOLD⁽⁸¹⁾ and GRAHAM-SMITH,⁽⁴⁰⁾ lag period in very small inoculation is longer than that in large inoculation.

In the present experiment, using *Pseudomonas fluorescens*, the effect of the initial number of bacterial cells introduced in the inoculum on the decomposition of fish muscle as well as the bacterial growth rate was studied.

Experiment.

Fifty cubic centimeters of the suspension of muscle of carangoid fish were made by means of the previously described method, put into a sterilized flask of a capacity of 200 c.c. and filled to 150 c.c. with distilled water and then sterilized. Broth culture of the organisms at 27°C for 48 hours was diluted to various degrees between 10 and 10⁶ times with sterilized distilled water. One cubic centimeter of these bacterial suspensions was added to the above flasks and incubated at 26°C–27°C. At definite intervals, volatile basic nitrogen in 10 c.c. of the mixtures and the number of living cells in 1 c.c. were measured.

Results.

Results obtained are given in Table 3 and are represented graphically in Figs. 2 and 3. In the Table, the generation time g and the velocity coefficient of the growth rate k were calculated by the commonly used following formulae (1), (2) and (3).

When the following abbreviations are used

t = Time (hour)

B = Initial number of bacteria (i.e., when $t = 0$)

b = Number of bacteria after time t

g = Average length of one generation time in time t

n = Number of generations in time t

It is evident that $n = t/g$.

If there is one organism at the beginning, there would be 2 ^{n} bacteria after n generations. If there were B bacteria at the beginning, there would be $B2^n$ bacteria at the end of n th generation, that is,

$$b = B2^n \quad \text{and} \quad b = B2^{\frac{t}{g}}$$

Solving for g

$$g = \frac{t \log 2}{\log b - \log B} \dots\dots\dots(1)$$

On the other hand, the rate of increase in the number of bacteria at any instant, that is, $\frac{db}{dt}$, varies directly with the number of bacteria. This may be written in the form,

$$\frac{db}{dt} = kb,$$

in which k is a constant and represents the velocity coefficient of the rate of growth.

Upon integration, this becomes

$$\ln b = kt + \text{constant of integration}$$

The constant of integration may be determined by evaluating b when $t = 0$. At this instant $b = B$, and the constant is therefore $\ln B$.

The equation then becomes

$$\ln \frac{b}{B} = kt$$

Solving for k

$$k = \frac{\ln b - \ln B}{t} \dots\dots\dots(2)$$

In another form this becomes

$$b = Be^{kt}$$

Since,

$$b = B2^n = B2^{\frac{t}{g}}$$

$$\therefore e^{kt} = 2^{\frac{t}{g}} \quad \text{and} \quad e^k = 2^{\frac{1}{g}}$$

$$\therefore k = \frac{\ln 2}{g} = \frac{0.694}{g} \dots\dots\dots(3)$$

The relationship between the generation time and the initial number of bacteria introduced in the inoculum is shown in Fig. 4. From this figure we learn clearly the dependence of the rate of growth on the initial number of inoculated bacteria, and that the velocity coefficient of growth rate k with smaller amount of inoculum is greater than that with larger one. But it will be also seen that the time required in reaching the asymptotic condition depends on the amount of inoculum, being slightly longer with smaller amount of inoculum, although, it is nearly the same irrespective of the initial number of organisms introduced in the inoculum.

The decomposition of muscle is clearly seen from Figs. 2 and 3, and is independent of the number of bacteria initially introduced, viz. the relation of the amount of volatile base produced against time is given by one and the same curve. After the number of living cells reached nearly the maximum, volatile base was quickly produced as previously experimented.

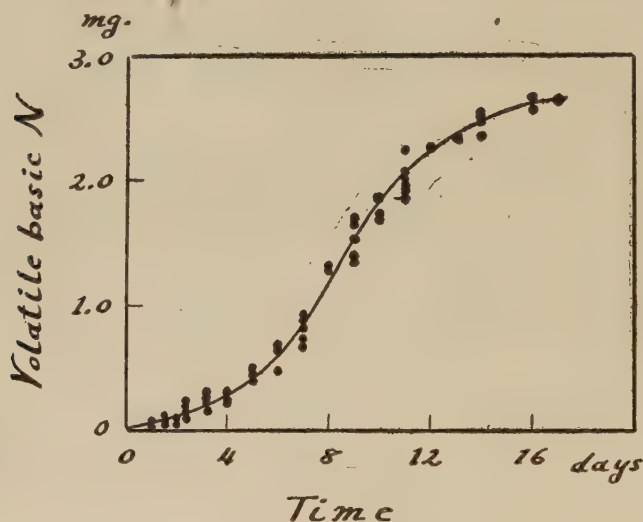


Fig. 2. Production of volatile basic nitrogen in the bacterial decomposition with different amounts of inoculum.

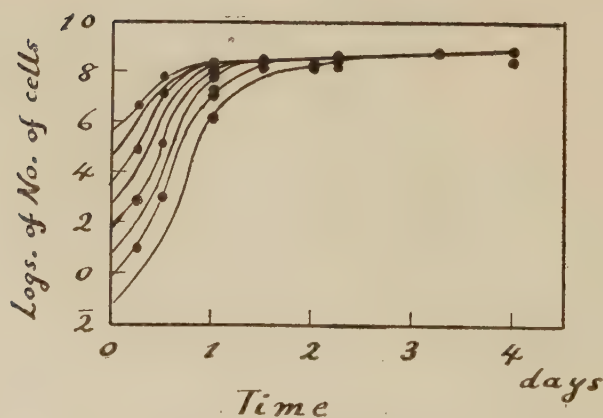
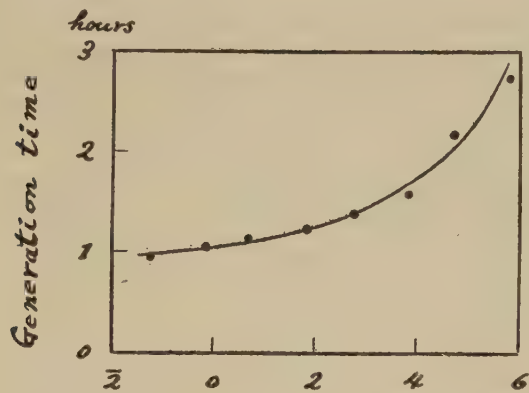


Fig. 3. Growth of bacteria in the case of different amounts of inoculum.



Log. of No. of bacteria at the beginning

Fig. 4. Relation between the generation time (average length of one generation time within 24 hours) and the initial number of inoculated bacteria.

Table 3. Decomposition of fish muscle and rate of growth of bacteria in relation to the number of organisms introduced in the inoculum.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increas.
days				mg.	mg.
0	652 × 10 ³			0.175	
1/4	372 × 10 ⁴	2.39	0.29	0.175	0
1/2	668 × 10 ⁵	1.33	0.52	0.175	0
1	283 × 10 ⁶	5.76	0.12	0.210	0.035
1.1/2	—			0.245	0.070
2.1/4	485 × 10 ⁶			0.406	0.231
3.1/4	740 × 10 ⁶			0.466	0.291
5	683 × 10 ⁶			0.571	0.396
7				1.092	0.917
8				1.505	1.330
9				1.855	1.670
12				2.436	2.261

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increas.
days				mg.	mg.
0	576 × 10 ²			0.175	
1	103 × 10 ⁶	2.22	0.31	0.193	0.018
2	239 × 10 ⁶	19.76	0.04	0.245	0.070
4	468 × 10 ⁶			0.490	0.315
6	—			0.858	0.683
9	—			1.855	1.680
11	—			2.254	2.079
14	530 × 10 ⁶			2.751	2.576
16	—			2.751	2.576

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	652×10^5			0.175	
1/4	654×10^5	1.81	0.38	0.175	0
1/2	156×10^5	0.76	0.91	0.175	0
1	970×10^5	4.55	0.15	0.210	0.035
1.1/2	—			0.228	0.053
2.1/4	583×10^6			0.399	0.224
5	659×10^6			0.588	0.413
7				1.057	0.882
8				1.495	1.320
10				2.048	1.873
11				2.380	2.205

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	576			0.175	
1	720×10^5	1.42	0.49	0.175	0
2	135×10^6	26.47	0.03	0.221	0.046
4	256×10^6			0.385	0.210
6	—			0.812	0.637
7	—			0.945	0.870
9	—			1.715	1.540
11	—			2.093	1.918
14	560×10^6			2.720	2.545
16	—			2.818	2.643

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	652*			0.175	
1/4	660	1.80	0.39	0.175	0
1/2	149×10^5	0.77	0.90	0.175	0
1	573×10^5	1.40	0.50	0.193	0.018
1.1/2	420×10^5			0.193	0.018
2.1/4	430×10^6			0.228	0.053
3.1/4	580×10^6			0.396	0.221
5	570×10^6			0.672	0.497
8	—			1.488	1.313
10				1.904	1.729
13				2.545	2.370

*—in 10 c.c.

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	576*			0.175	
1	148×10^5	1.13	0.61	0.186	0.011
2	188×10^6	6.54	0.11	0.238	0.063
4	342×10^6			0.385	0.210
7	—			0.833	0.658
9	—			1.575	1.400
11	—			2.065	1.890
14	630×10^6			2.685	2.510
17	—			2.853	2.678

*—in 100 c.c.

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	652*			0.175	
1/4	8	1.66	0.42	0.175	0
1/2	930	0.87	0.80	0.175	0
1	780×10^4	0.97	0.75	0.175	0
1.1/2	120×10^6			0.193	0.018
2.1/4	470×10^6			0.263	0.088
3.1/4	841×10^6			0.361	0.186
5	—			0.665	0.490
6	635×10^6			0.826	0.651
7	—			1.050	0.875
10				1.880	1.705
11				2.076	1.901

*—in 1000 c.c.

Table 3. continued.

Time of incubation	Bacteria			Volatile bas. N	
	No. of cells	<i>g</i>	<i>k</i>	Determ.	Increases.
days				mg.	mg.
0	57*			0.175	
1	127×10^4	0.98	0.71	0.175	0
2	131×10^6	3.59	0.19	0.210	0.035
4	202×10^6			0.378	0.203
6	—			0.630	0.455
7	—			0.875	0.700
9	—			1.523	1.343
11	—			2.030	1.855
14	448×10^6			2.538	2.363

*—in 1000 c.c.

III. Progress of Bacterial Decomposition of Extractive Matter and Rate of Bacterial Growth in Relation to the Concentration of the Extractive Matter of Meat of Aquatic Animals.

When with respect to the process of bacterial decomposition, various kinds of extractive matter of meats are compared with each other, the solution of all kinds must be in the same concentration, and various concentrations of the same kind of solution must also be studied, if the process of the decomposition varies with the concentration of solution. But the equally concentrated solutions can not be prepared from various kinds of meat extracts having different compositions. Although the solution can be so prepared as a certain material (for example total nitrogen) among various components may become in the same concentration, it is very troublesome and seems, therefore, to be unfit, if many kinds of materials which may easily be decomposed must be treated at the same time.

The prime object of the present experiment is to examine how the process of the bacterial decomposition is affected by different concentrations in the same kind of the solution of meat extract, and moreover, it aims to determine the suitable standard to be used for comparing the progresses of the bacterial decomposition among many kinds of meat extracts. The growth rates of bacteria in media containing various quantities of the meat extract were observed in this connection.

So far as the present author is aware, little is known with regard to the process of the bacterial decomposition of medium. MIYAKE⁽⁷⁵⁾ found that ammonification and nitrification are due to the monomolecular autocatalytic chemical reaction in the soil. PULLEY and GREAVES⁽⁸⁴⁾ have applied the differential equation which expresses the speed of a monomolecular autocatalytic chemical reaction to the process of nitrate and carbon-dioxide accumulation by soil bacteria and yeast. Again, our knowledge of the relation between the rate of growth of bacteria and the concentration of food in medium is comparatively few. According to PENFOLD and NORRIS,⁽⁸²⁾ the generation time of *Bact. typhosum* in peptone water remains practically constant, if the peptone concentration exceeds 0.4%.

Experiment 1. Decomposition by *Pseudomonas fluorescens* in the meat extract of chub mackerel.

The solution of meat extract of chub mackerel was prepared by

the method already described. Certain volume of this solution was put into a sterilized flask of the capacity of 300 c.c. and diluted to various concentrations with physiological salt solution as given in Table 4, and sterilized. To the flask 0.1 c.c. of the bacterial suspension was added, which was broth culture (at 26°C–27°C for 48 hours) diluted to 1,000 times with sterilized distilled water, and was incubated at 26°C–27°C. At certain definite intervals, formol titrating nitrogen

Table 4. Composition of the decomposing meat extract's solution. (by *Pseudomonas fluorescens*).

No. of flask	I	II	III	IV	V	VI	VII
Solution of meat extract	140 c.c.	70	40	20	10	5	2
Physiological salt solution	0 c.c.	70	100	120	130	135	138

(amino and ammonium nitrogen) in 5 c.c. and bacterial counts in 1 c.c. of the the mixture were determined.

Table 5. Growth of Bacteria. (*Pseudomonas fluorescens*).

Time of incubation	I	II	III	IV	V	VI	VII
0 h.	537	537	537	537	537	537	537
8	837×10^2	631×10^2	144×10^3	136×10^3	641×10^2	198×10^2	413×10
24	298×10^6	312×10^6	397×10^6	438×10^6	298×10^6	162×10^5	—
48	461×10^6	489×10^6	536×10^6	416×10^6	248×10^6	734×10^5	508×10^4
4 d.	334×10^6	324×10^6	328×10^6	318×10^6	284×10^6	281×10^6	118×10^6

Table 6. Production of amino and ammonium nitrogen. (by *Pseudomonas fluorescens*).

Time of incubation	I		II		III		IV		V		VI		VII	
	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	1.785	—	0.910	—	0.508	—	0.245	—	0.123	—	0.063	—	0.028	—
1	1.785	0	—	—	—	—	0.245	0	0.123	0	0.063	0	0.028	0
2	1.995	0.210	1.155	0.245	0.701	0.193	0.368	0.123	0.123	0	0.123	0.060	0.063	0.035
3	2.380	0.595	1.400	0.490	0.823	0.315	0.420	0.175	0.193	0.070	0.133	0.070	0.070	0.042
4	3.045	1.260	1.610	0.700	0.911	0.403	0.455	0.210	0.221	0.098	0.140	0.077	0.077	0.049
5	—	—	1.785	0.875	0.963	0.455	0.473	0.228	0.239	0.116	0.144	0.081	0.081	0.053
6	3.640	1.855	1.855	0.945	—	—	0.490	0.245	0.246	0.123	0.144	0.081	0.081	0.053
7	3.710	1.925	1.890	0.980	0.998	0.490	0.508	0.263	0.253	0.130	0.144	0.081	0.081	0.053
8	3.710	1.925	1.890	0.980	0.998	0.490	0.508	0.263	0.253	0.130	—	—	—	—

Results obtained are given in Tables 5 and 6, and also in Figs. 5 and 6.

In a very dilute solution of extractive matter, the growth rate of bacteria increases with the increase of concentration, but it does not show any increase when the concentration is higher than a certain definite limit, i. e. in this experiment, the growth

rate is nearly the same when the quantity of total nitrogen in 5 c.c. is above 0.854 mg.

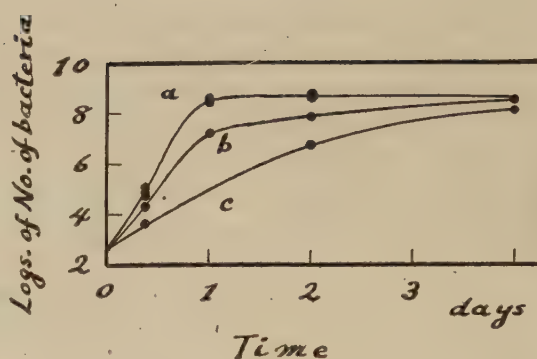


Fig. 5. Bacterial growth in the solution of various concentrations of meat extract of chub mackerel.

a.....I, II, III, IV, V.
b.....VI.
c.....VII.

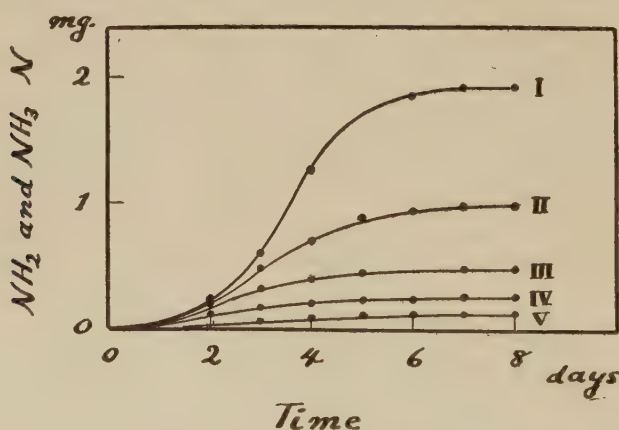


Fig. 6. Production of amino and ammonium nitrogen from the bacterial decomposition in various concentrations of meat extract of chub mackerel.

PULLEY and GREAVES⁽⁸⁴⁾ have applied the equation which expresses the speed of a monomolecular autocatalytic chemical reaction to nitrate and carbon-dioxide accumulation. The equation can be applied to the data on the production of formol titrating nitrogen (amino and ammonium nitrogen) in the bacterial decomposition of the solution of meat extract.

The equation implies that the catalyst is produced by the reaction in exactly the same proportion as the end-product y . It then becomes one of the reacting substances. Denoting by a the original concentration of the substrate, by y the amount used by the reaction (the product) and also, by the assumption, the catalyst, and by k_1 proportionality constant, the rate of reaction may be expressed as

$$\frac{dy}{dt} = k_1 y(a-y) \dots\dots\dots(1)$$

However, the amount of the original substrate a is not generally the limiting factor of reaction, but the reaction must be considered reversible, and an equilibrium is reached which is expressed by subtracting the reverse from the forward reaction.

$$\frac{dy}{dt} = k_1 y(a-y) - k_2 y^2 \dots\dots\dots(2)$$

It is understood that the catalyst is active in both directions. A second constant k_2 must be introduced for the reverse reaction. By a little manipulation and substituting A for $\frac{k_1 a}{k_1 - k_2}$, we get

$$\frac{dy}{dt} = y \frac{k_1 a}{A} (A-y) \dots\dots\dots(3)$$

which is, since $\frac{k_1 a}{A}$ is a constant, identical with (1). But A is a fractional part of a and defines the limit of maximum amount of end-product due to equilibrium between the forward and reverse reactions rather than total amount of nutrient substrate originally present.

Integrating (3) and substituting K for $\frac{k_1 a}{2.3A}$, we get

$$\log \frac{y}{A-y} = Kt + C$$

C is evaluated as $-Kt_1$ where t_1 is the time at which the reaction is half completed, that is, $y = \frac{A}{2}$, and we obtain

$$\log \frac{y}{A-y} = K(t-t_1)$$

The curve is symmetrical with respect to its center at $y = \frac{A}{2}$, $t = t_1$.

When solved for y , it gives

$$y = \frac{A \cdot 10^{K(t-t_1)}}{1 + 10^{K(t-t_1)}}$$

In applying this equation to experimental data the three constants, A , K and t_1 must be properly evaluated. The constant A , representing the amount of end-product, is evaluated by inspection after plotting the data. Both K and t_1 can be calculated from the graph of the equation.

$\log \frac{y}{A-y}$, plotted against time in the diagram obtained from the data in the Table 6 (Fig. 6) is linear as shown in Fig. 7. So, the above equation seems to be applicable to the process of the bacterial decomposition of meat extract.

The values of K and t_1 calculated from Fig. 7 are given in Table 7, and the former becomes slightly larger with increasing concentration, though degree of the increase is very small and becomes only slightly larger even with very much increased concentration. The value of t_1 becomes larger with increasing concentration.

The value of A/a remains practically constant except at very dilute concentration, that is, above a certain definite concentration as shown in Table 7. The value of a is the total nitrogen (exclusive of the formol titrating nitrogen already present) which is determined by means of KJELDAHL's method.

Table 7. Values of various constants. (by *Pseudomonas fluorescens*).

No. of flask	Total N	a	A	A/a	K	t_1
	mg.	mg.	mg.			d.
I	12.025	10.240	1.925	0.188	0.57	3.60
II	6.030	5.120	0.980	0.191	0.50	3.00
III	3.434	2.926	0.490	0.168	0.48	2.50
IV	1.708	1.463	0.263	0.179	—	—
V	0.854	0.731	0.130	0.178	—	—
VI	0.428	0.365	0.081	0.222	—	—
VII	0.184	0.146	0.053	0.363	—	—

Experiment 2. Decomposition by *Serratia marcescens* in the meat extract of chub mackerel.

Certain volume of the original solution of meat extract of chub mackerel was put into a sterilized flask of the capacity of 200 c.c. and diluted to various concentrations with physiological salt solution as shown in Table 8. To the flask 0.1 c.c. of the bacterial suspension was added, which was broth culture (at 26°C–27°C for 48 hours)

Table 8. Composition of the decomposing meat extract's solution. (by *Serratia marcescens*).

No. of flask	I	II	III	IV	V	VI	VII
Solution of meat extract	100 c.c.	50	25	12.5	7.5	4	2
Physiological salt solution	0 c.c.	50	75	87.5	92.5	96	98

diluted to 1,000 times with sterilized distilled water, and incubated at 26°C–27°C, and then the number of living cells and formol titrating nitrogen were measured just as in the Experiment 1.

Results obtained are shown in Tables 9 and 10.

Table 9. Growth of bacteria (*Serratia marcescens*).

Time of incubation	I	II	III	IV	V	VI	VII
0 h.	614	614	614	614	614	614	614
8	118×10^4	181×10^4	214×10^4	203×10^4	207×10^4	208×10^4	981×10^3
24	842×10^6	872×10^6	594×10^6	563×10^6	448×10^6	297×10^6	148×10^6
3 d.	224×10^7	239×10^7	182×10^7	123×10^7	484×10^6	281×10^6	243×10^6

The rate of the bacterial growth is nearly the same for various concentrations, though it is much smaller at exceedingly low concentration of the extractive matter as evidently seen from Table 9.

Table 10. Production of amino and ammonium nitrogen. (by *Serratia marcescens*).

Time of incubation	I		II		III		IV		V		VI		VII	
	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	2.135		1.068		0.525		0.263		0.158		0.088		0.035	
1	2.170	0.035	1.121	0.053	0.585	0.060	0.316	0.053	0.211	0.053	0.158	0.070	0.105	0.070
3	2.345	0.210	1.401	0.333	0.840	0.315	0.526	0.263	0.351	0.193	0.193	0.105	0.140	0.105
4	2.520	0.385	1.611	0.543	0.980	0.455	0.578	0.315	0.351	0.193	0.211	0.123	0.140	0.105
5	3.010	0.875	1.891	0.823	1.030	0.505	0.578	0.315	0.351	0.193	0.211	0.123	—	—
7	4.025	1.890	2.171	1.103	1.085	0.560	0.578	0.315	—	—	—	—	—	—
8	4.340	2.205	2.171	1.103	—	—	—	—	—	—	—	—	—	—
9	4.375	2.240	—	—	—	—	—	—	—	—	—	—	—	—

Relation between $\log \frac{y}{A-y}$ and time is obtained from the data given in Table 10, and it is linear as shown in Fig. 8. To the process of the formation of formol titrating nitrogen from the decomposition

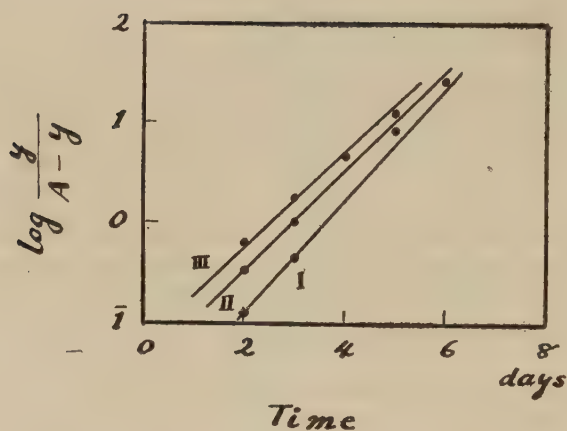


Fig. 7. Relation between $\log \frac{y}{A-y}$ and time, in the case of the bacterial decomposition (by *Pseudomonas fluorescens*) of various concentrations of meat extract of chub mackerel.

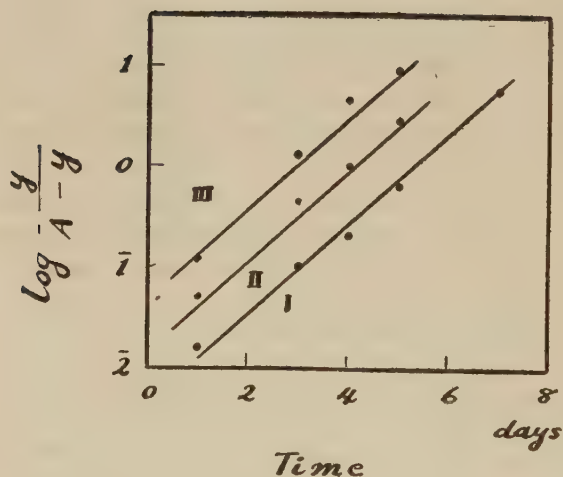


Fig. 8. Relation between $\log \frac{y}{A-y}$ and time, in the case of the bacterial decomposition (by *Serratia marcescens*) of various concentrations of meat extract of chub mackerel.

Table 11. Values of various constants. (by *Serratia marcescens*).

No. of flask	Total N	<i>a</i>	<i>A</i>	<i>A/a</i>	<i>K</i>	<i>t</i> ₁
	mg.	mg.	mg.			d.
I	13.900	11.765	2.240	0.190	0.45	5.30
II	6.951	5.883	1.103	0.188	0.45	4.10
III	3.475	2.950	0.560	0.190	0.45	3.00
IV	1.738	1.475	0.298	0.201	—	—
V	1.043	0.885	0.193	0.218	—	—
VI	0.557	0.469	0.123	0.262	—	—
VII	0.278	0.243	0.105	0.432	—	—

by *Serratia marcescens* the law of monomolecular autocatalytic reaction can thus be applied.

The values of K and t_1 calculated from Fig. 8 are shown in Table 11. From this Table it is clear that K is the same, independent of the concentration, while t_1 becomes larger with the increasing concentration of meat extract. A/a is nearly the same above a certain definite concentration of extractive matter as shown in Table 11.

Experiment 3. Decomposition by *Pseudomonas fluorescens* in the meat extract of halibut.

The original solution of meat extract of halibut was prepared by

Table 12. Growth of bacteria. (*Pseudomonas fluorescens*).

Time of incubation	I	II	III	IV	V	VI	VII
0 h.	723	723	723	723	723	723	723
8	146×10^2	126×10^2	141×10^2	710×10	411×10	176×10	862
24	139×10^6	973×10^5	748×10^5	482×10^5	469×10^5	831×10^4	793×10
48	351×10^6	216×10^6	211×10^6	713×10^5	554×10^5	356×10^5	641×10^4
3 d.	108×10^7	672×10^6	924×10^6	248×10^6	253×10^6	844×10^5	563×10^5

Table 13. Production of amino and ammonium nitrogen. (by *Pseudomonas fluorescens*).

[illegible]

the method described already. This experiment was carried out by the same method as the Experiment 2 excepting the organism employed. Organism employed was *Pseudomonas fluorescens* which was broth culture at 26°C–27°C for 48 hours and was used in the same manner as in the Experiment 1.

Results obtained are given in Tables 12 and 13. From the Table 13, Figs. 9 and 10 are plotted. These results are evidently in accord with the findings in the Experiment 1 and 2. K , t_1 and A/a are given in Table 14.

Table 14. Values of various constants. (by *Pseudomonas fluorescens*).

No. of flask	Total N	a	A	A/a	K	t_1
	mg.	mg.	mg.			d.
I	8.400	7.490	0.595	0.080	0.213	9.90
II	4.200	3.745	0.315	0.084	0.203	7.70
III	2.100	1.873	0.193	0.102	0.194	5.60
IV	1.060	0.936	0.140	0.150	—	—
V	0.633	0.563	0.105	0.187	—	—
VI	0.353	0.300	0.070	0.233	—	—
VII	0.185	0.150	0.053	0.354	—	—

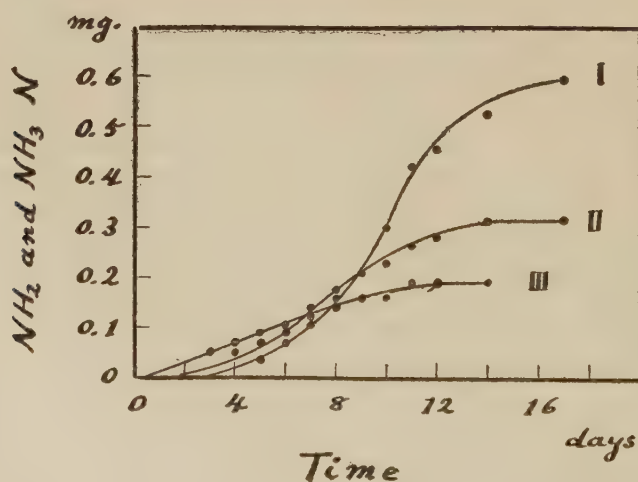


Fig. 9. Production of amino and ammonium nitrogen from the bacterial decomposition (by *Pseudomonas fluorescens*) in various concentrations of meat extract of halibut.

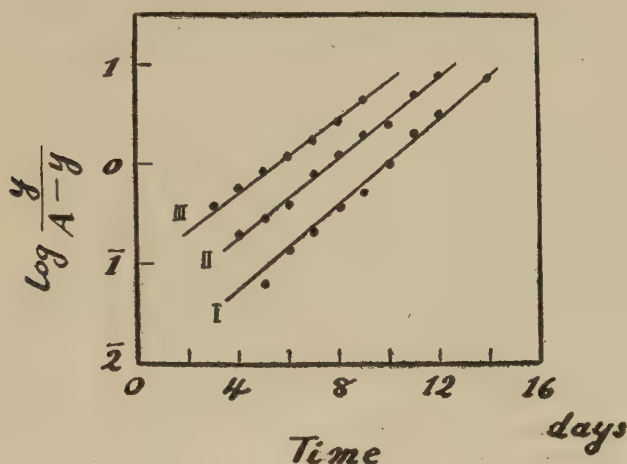


Fig. 10. Relation between $\log \frac{y}{A-y}$ and time, in the case of the bacterial decomposition (by *Pseudomonas fluorescens*) of various concentrations of meat extract of halibut.

Experiment 4. Decomposition by *Pseudomonas fluorescens* in the meat extract of squid.

The solution of meat extract of squid was prepared. The present experiment was carried out by the same manner of the Experiment 3.

Results obtained are shown in Tables 15 and 16. Relation between $\log \frac{y}{A-y}$ and time was obtained from the data in Table 16 as shown in Fig. 11. K , t_1 and A/a calculated from Fig. 11 or Table 16 are given in Table 17.

Table 15. Growth of bacteria. (*Pseudomonas fluorescens*).

Time of incubation	I	II	III	IV	V	VI	VII
0 h.	768	768	768	768	768	768	768
8	—	—	104×10^2	108×10^2	120×10^2	105×10^2	573×10
24	180×10^6	—	327×10^6	282×10^6	148×10^6	631×10^5	376×10^6
48	261×10^7	259×10^7	268×10^7	119×10^7	427×10^6	176×10^6	441×10^5
3 d.	582×10^7	392×10^7	321×10^7	206×10^7	584×10^6	320×10^6	654×10^5

Table 16. Production of amino and ammonium nitrogen. (by *Pseudomonas fluorescens*).

Time of incubation	I		II		III		IV		V		VI		VII	
	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y	Detm.	y
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0	3.500	—	1.750	—	0.875	—	0.438	—	0.263	—	0.140	—	0.070	—
2	—	—	1.890	0.140	—	—	0.543	0.105	0.316	0.053	—	—	—	—
3	3.850	0.350	2.013	0.263	1.155	0.280	0.596	0.158	0.351	0.088	—	—	—	—
4	4.200	0.700	2.143	0.385	1.225	0.350	0.666	0.228	0.368	0.105	—	—	—	—
5	4.585	1.085	2.275	0.525	1.330	0.455	0.683	0.245	0.386	0.123	0.228	0.088	—	—
6	5.075	1.575	2.520	0.770	—	—	0.718	0.280	0.421	0.158	0.263	0.123	0.123	0.053
7	—	—	—	—	—	—	—	—	0.421	0.158	0.263	0.123	0.123	0.053
8	5.600	2.100	2.765	1.015	—	—	0.718	0.280	—	—	—	—	—	—
9	—	—	2.835	1.085	—	—	—	—	—	—	—	—	—	—
10	—	—	2.870	1.120	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	1.435	0.560	—	—	—	—	—	—	—	—
12	5.698	2.198	—	—	—	—	—	—	—	—	—	—	—	—

Table 17. Values of various constants. (by *Pseudomonas fluorescens*).

No. of flask	Total N	a	A	A/a	K	t ₁
	mg.	mg.	mg.			d.
I	16.450	12.950	2.198	0.169	0.410	4.85
II	8.225	6.475	1.120	0.173	0.330	4.75
III	4.113	3.238	0.560	0.173	0.310	3.15
IV	2.057	1.619	0.280	0.173	—	—
V	1.234	0.971	0.158	0.168	—	—
VI	0.657	0.517	0.123	0.238	—	—
VII	0.329	0.259	0.053	0.204	—	—

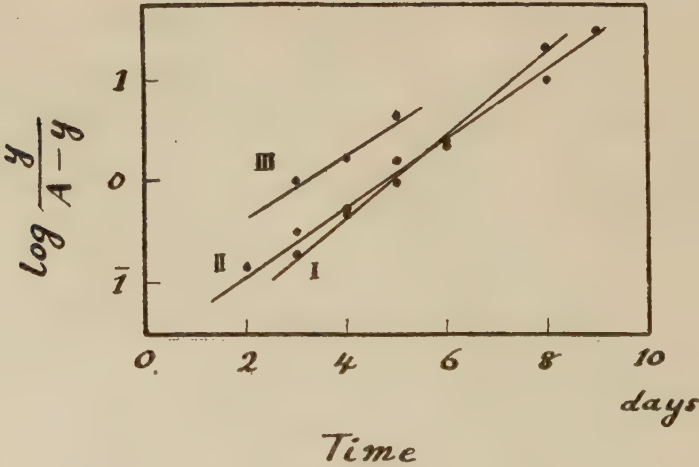


Fig. 11. Relation between $\log \frac{y}{A-y}$ and time, in the case of the bacterial decomposition (by *Pseudomonas fluorescens*) of various concentrations of meat extract of squid.

It appears that these results obtained have the same tendency as the preceding ones.

Discussion.

From the results of all of the experiments carried out, it can be concluded that the rate of growth of bacteria in the solution of extractive matter of meat of aquatic animals remains practically constant, provided the concentration of these matter is not very dilute. Thus, there are no effect of the concentration on the rate of bacterial growth within the range of the concentration used for usual experiments.

The progress of formation of formol titrating nitrogen (amino and ammonium nitrogen) in the bacterial decomposition of meat extract of aquatic animals can be defined by the equation

$$\log \frac{y}{A-y} = K(t-t_1)$$

which is derived from the equation expressing the speed of a monomolecular autocatalytic chemical reaction:

$$\frac{dy}{dt} = ky(a-y)$$

It is suggested, though from limited data, that one cycle of evolution follows another, the value of y in the curve being calculated by summing up the several separate values at any given time.

In the equation there are two variables y , the quantity of the formol titrating nitrogen, and t the time, together with three constants A , K and t_1 . For meat extract of a definite animal the constant K becomes larger with increasing concentration of the solution, though slightly, increase being only very slight even with the increase in the concentration of several times. It is acceptable, therefore, that in the case of a definite animal the value of K is practically almost indifferent to increasing or decreasing concentration of the solution within the range usually experimented. The constant t_1 is influenced by the concentration and becomes larger with increasing concentration of the solution.

From the above mentioned facts it is evident that in the case of the bacterial decomposition of the same kind of meat extract, the speed of the decomposition is almost irrespective of the concentration of the solution, but the period required for the decomposition, in other words, inductive period (or lag period), becomes longer with increasing concentration of meat extract.

The constant A must be influenced by increasing or decreasing concentration for the same kind of meat extract, because A is a fractional part of a , representing the original concentration of the substrate. However, the ratio of the total nitrogen (exclusive of the formol titrating nitrogen already present) to the maximum amount of the formol titrating nitrogen produced, i. e. A/a is nearly the same irrespective of the concentration for the same kind of animal, provided the meat extract is not very dilute.

Eventually, it can be concluded that even in the case of the bacterial decomposition of various kinds of meat extracts, the speed of the decomposition can be compared by the value of K , and the final rate of the decomposition can be compared by the value of A/a , and at the same time the value of t_1 must be also concerned.

Appendix.

From the preceding experimental results (Table 3 and Fig 2 in Chapter II), the relation between $\log \frac{y}{A-y}$ and time was obtained in

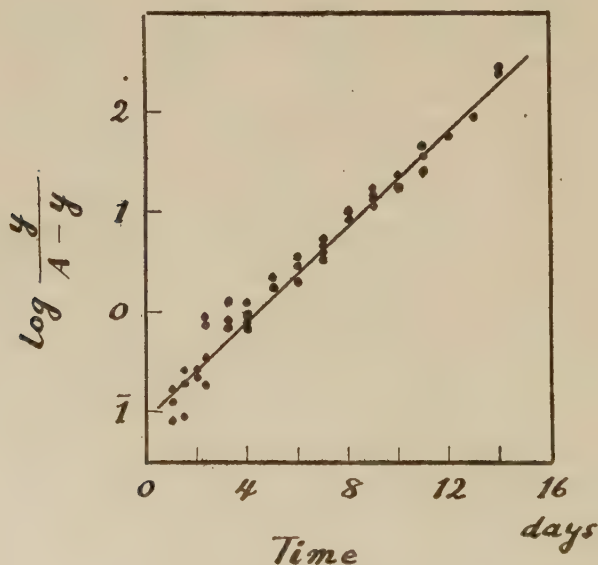


Fig. 12. Relation between $\log \frac{y}{A-y}$ and time, in the case of the bacterial decomposition of fish muscle (from Table 3 and Fig. 2).

linear form as shown in Fig. 12. It is evident that the progress of the production of volatile basic nitrogen in the bacterial decomposition of fish muscle can be defined by the equation of the form

$$\log \frac{y}{A-y} = K(t-t_1)$$

and therefore, even in the case of muscles, it can be held that the progress of the bacterial decomposition is characterised by the three constants, K , A and t_1 , just as in the case of extracts.

IV. The Velocity and the Final Rate of the Bacterial Decomposition of Meat Extract of Several Kinds of Aquatic Animals.

So far as the present author is aware, nothing is known about the process of bacterial decomposition of meat extract of aquatic animals.

The present experiment aims to ascertain the final rates and the velocities of bacterial decomposition of meat extracts as well as the growth rate of the bacteria in the solution of them, with the following kinds of aquatic animals: bonito, carangoid, chub mackerel, serranid, sea bream, halibut and spiny lobster.

The velocities of the decomposition were compared by the value of K , and the final rates by the value of A/a according to the conclusions of Chapter III.

Experiment.

The solutions of meat extract of the above mentioned animals were prepared by the method previously described. One hundred and fifty cubic centimeters of these solution and 150 c.c. of these solutions diluted to twice the volume with physiological salt solution were put into a flask of the capacity of 300 c.c. and sterilized. To each flask 0.1 c.c. of the bacterial suspension was added, which was broth culture of *Pseudomonas fluorescens* (at 26–27°C for 48 hours) diluted to 1,000 times with sterilized distilled water, and incubated at 26–27°C. At definite intervals, amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. were determined.

Results and Discussion.

Results obtained are shown in Tables 18–25.

From the Table 18 it seems that the growth of bacteria in the

Table 18. Growth of bacteria.

Kind of extract	0 h.	22 hs.	48 hs.
Bonito	335	289×10^5	568×10^6
Chub mackerel		396×10^5	937×10^6
Carangoid.....		482×10^5	109×10^7
Serranid		314×10^5	564×10^6
Sea bream.....		524×10^5	688×10^6
Halibut		382×10^5	518×10^6
Spiny lobster		387×10^5	682×10^6

solution of meat extract is almost indifferent to the kind of animals employed.

Table 19. Bonito.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	3.238			1.663		
2	3.238	0		—	—	—
3	3.238	0		1.926	0.263	1.153
5	3.676	0.438	1.109	2.713	1.050	0.038
6	4.026	0.788	1.405	3.151	1.488	0.453
7	4.921	1.663	1.882	3.501	1.838	1.021
8	5.621	2.363	0.203	3.676	2.013	
9	6.651	3.413	0.893	3.676	2.013	
13	7.088	3.850		3.676	2.013	
14	7.088	3.850		—	—	

Table 20. Chub mackerel.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.838			0.963		
2	1.838	0		1.086	0.123	1.204
3	1.888	0.350	1.399	1.313	0.350	1.810
5	2.976	1.138	0.270	1.663	0.700	0.560
6	3.238	1.400	0.602	1.751	0.788	0.876
7	3.501	1.663	1.279	—	—	—
8	—	—	—	1.856	0.893	
9	3.588	1.750		1.856	0.893	
10	3.588	1.750		—	—	

Table 21. Halibut.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.875	—		0.438	—	
4	—	—		0.438	0	
5	—	—		0.438	0	
6	0.875	0		0.508	0.070	1.425
7	—	—		0.543	0.105	1.664
8	0.980	0.105	1.316	0.631	0.193	0.141
10	1.120	0.245	1.825	0.701	0.263	0.574
13	1.488	0.613		0.771	0.333	
14	1.488	0.613		0.771	0.333	

Table 22. Sea bream.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.875	—		0.438	—	
3	—	—		0.438	0	
5	0.875	0		0.438	0	
6	0.875	0		0.526	0.088	1.551
7	0.945	0.070	1.110	0.543	0.105	1.664
8	0.980	0.105	1.316	0.631	0.193	0.141
10	1.173	0.298	1.979	0.701	0.263	0.574
13	1.488	0.613		0.771	0.333	
14	1.488	0.613		0.771	0.333	

Table 23. Serranid.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.840	—		0.420	—	
3	0.840	0		0.420	0	
5	0.840	0		0.420	0	
6	0.840	0		0.525	0.105	1.574
7	0.945	0.105	1.223	0.560	0.140	1.758
8	0.980	0.140	1.372	0.630	0.210	0.080
10	1.138	0.298	1.834	0.700	0.280	0.426
13	1.575	0.735		0.805	0.385	
14	1.575	0.735		0.805	0.385	

Table 24. Carangoid.

Time of incubation	Original solution		
	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.	
0	1.068		
2	1.226	0.158	1.424
3	1.401	0.333	1.900
5	1.663	0.595	0.577
7	1.821	0.753	
8	1.821	0.753	

Table 25. Spiny lobster.

Time of incubation	Original solution			Diluted solution (to 2 times)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	5.688			2.888		
2	6.038	0.350	1.083	3.326	0.438	1.554
3	7.176	1.488	1.930	4.026	1.138	0.348
5	8.576	2.888	0.918	4.463	1.575	1.256
6	8.786	3.098	1.346	4.516	1.628	1.668
7	8.891	3.203	1.962	—	—	
8	8.926	3.238		4.551	1.663	
9	8.926	3.238		4.551	1.663	

Table 26. Values of various constants.

Kind of meat extract	Original solution					
	Total N	a	A	A/a	t ₁	K
	mg.	mg.	mg.		d.	
Bonito	15.575	12.337	3.850	0.312	7.20	0.48
Chub mackerel	10.150	8.312	1.750	0.211	4.65	0.48
Carangoid	8.050	6.982	0.735	0.108	3.35	0.385
Serranid	8.470	7.630	0.735	0.096	10.40	0.25
Sea bream	7.700	6.825	0.613	0.090	10.40	0.25
Halibut	8.225	7.350	0.613	0.083	10.40	0.25
Spiny lobster	14.000	8.312	3.238	0.390	3.25	0.52

Diluted solution (to 2 times)						
Bonito	7.788	6.169	2.103	0.326	4.95	0.48
Chub mackerel	5.075	4.156	0.857	0.206	3.90	0.47
Serranid	4.235	3.815	0.385	0.101	7.80	0.25
Sea bream	3.850	3.413	0.333	0.098	7.80	0.25
Halibut	4.113	3.675	0.333	0.091	7.80	0.25
Spiny lobster	7.000	4.156	1.663	0.400	2.45	0.50

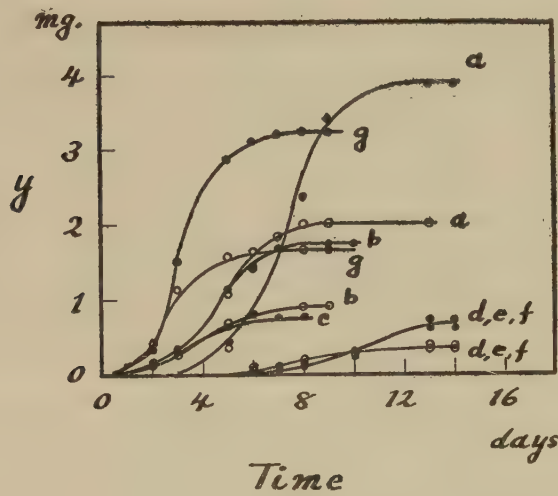


Fig. 13. Bacterial decomposition of meat extract of several kinds of aquatic animals.

a.....bonito, b.....chub mackerel, c.....carangoid, d.....halibut, e.....sea bream, f.....serranid, g.....spiny lobster.

●.....original solution.
○.....diluted solution to 2 times.

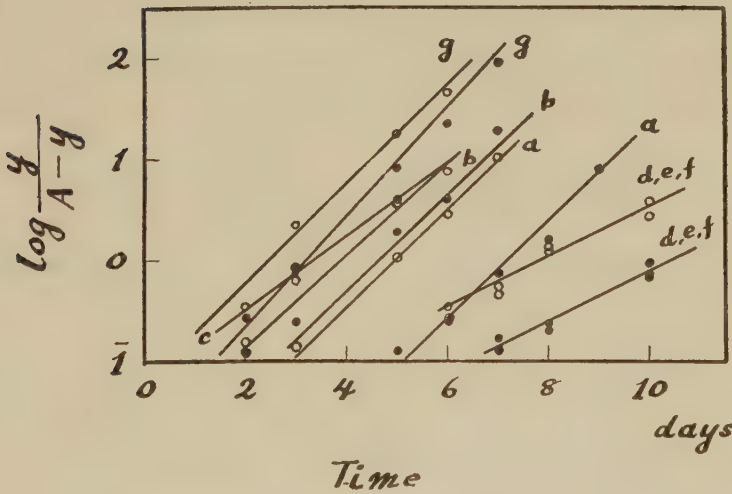


Fig. 14. Relation between $\log \frac{y}{A-y}$ and time. a, b, c, etc. and marks are the same in Fig. 13.

The relation between y , the decomposition product and t , the time can be obtained from Tables 19-25 and is graphically shown in Figs.

13 and 14. The values of K and t_1 can be calculated from Fig. 14, as are given in Table 26.

From the Table 26, it is clear that both the values of K and of A/a at two different concentrations of meat extract of a definite animal are nearly the same, but the value of t_1 is larger at higher concentration, as in the experiment of Chapter III. Therefore, the velocities of the bacterial decomposition of meat extract of several kinds of aquatic animals are capable to be compared by the value of K and also the final rates of the decomposition, by the value of A/a . When the value of K is large, also the value of A/a is large. These values depend on the kind of aquatic animals and the former arranged in descending order is as follows:

Spiny lobster, bonito, chub mackerel, carangoid, serranid, sea bream and halibut. Moreover, these may be divided into 4 grades, i.e. (1) spiny lobster, (2) bonito and chub mackerel, (3) carangoid, (4) serranid, sea bream and halibut.

From these facts it is presumed that, in the case of fishes, the final rates and the velocities of the bacterial decomposition of extractive matter of red meats such as bonito and chub mackerel are the greatest, those of white meats such as serranid, sea bream and halibut are the smallest, and those of the meat of carangoid are intermediate, and that those of crustacea such as spiny lobster are greater than those of fishes.

It is supposed that these facts play an important rôle on the rate and the velocity of the bacterial decomposition of an aquatic animal.

V. Bacterial Decomposition of the Mixture of Two Kinds of Meat Extracts.

We have previously studied (Chapter IV) that the final rate and the velocity of the bacterial decomposition of meat extract vary with the kind of aquatic animals.

The present experiment reveals author's observations on the final rate and the velocity of the bacterial decomposition of the mixtures in various concentrations of two kinds of the meat extracts, and on the growth of bacteria in the solutions of the mixture.

Experiment.

Chub mackerel (i.e. red meat fish) and halibut (i.e. white meat fish) were used. The solutions of meat extract of above two kinds of aquatic animals were prepared by the method as previously described.

Various quantities of these solutions were pipetted into a flask of a capacity of 200 c.c. and mixed as shown in Table 27 and sterilized. To each flask 0.1 c.c. of the bacterial suspension was added, which

Table 27. Composition of the mixtures.

No. of flask	I	II	III	IV	V	VI	VII	VIII	IX
Solution of meat extract of bonito	10 c.c.	25	50	75	90	0	100	0	50
Solution of meat extract of halibut	90 c.c.	75	50	25	10	100	0	50	0
Physiological salt solution	0 c.c.	0	0	0	0	0	0	50	50

was broth culture of *Pseudomonas fluorescens* (at 26°C–27°C for 48 hours) diluted to 1,000 times with sterilized distilled water, and incubated at 26°C–27°C. At definite intervals amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. were determined.

The flasks, numbered as VI, VII, VIII, IX are control.

Results and Discussion.

Results obtained are given in Tables 28 and 29. Figs. 15 and 16 are obtained from the data in Table 28.

Table 28. Decomposition of the mixtures.

Time of incubation	I			II		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.418			1.470		
2	1.418	0		1.488	0.018	2.272
4	1.418	0		1.558	0.088	2.995
6	1.471	0.053	2.838	1.628	0.158	1.284
8	1.576	0.158	1.376	1.820	0.350	1.745
9	1.646	0.228	1.584	1.995	0.525	0.062
11	1.926	0.508	0.208	2.188	0.718	0.438
13	—	—	—	2.363	0.893	1.012
14	2.188	0.770	1.163	—	—	—
15	—	—	—	2.450	0.980	—
17	2.241	0.823	—	—	—	—

Relation between $\log \frac{y}{A-y}$ and the time is almost linear. There-

Table 28. continued.

Time of incubation	III			IV		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.523			1.575		
2	1.663	0.140	1.097	1.785	0.210	1.204
4	1.926	0.403	1.674	2.258	0.683	1.911
6	2.363	0.840	0.302	2.800	1.225	0.614
8	2.643	1.120	0.904	2.975	1.400	1.056
9	2.713	1.190	1.231	3.028	1.453	1.318
11	2.783	1.260		3.098	1.523	
13	2.783	1.260		3.098	1.523	

Table 28. continued.

Time of incubation	V			VI		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.610			1.400		
2	1.855	0.245	1.222	—	—	
4	2.433	0.823	1.966	—	—	
6	3.115	1.505	0.857	1.435	0.035	2.722
8	3.255	1.645	1.372	1.470	0.070	1.047
9	3.325	1.715		1.505	0.105	1.247
11	3.325	1.715		1.680	0.280	1.824
13	—	—		1.925	0.525	0.478
14	—	—		2.013	0.613	0.849
17	—	—		2.100	0.700	

Table 28. continued.

Time of incubation	VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.645			0.700		
2	1.820	0.175	1.018	0.700	0	
4	2.520	0.875	1.951	—	—	
6	3.290	1.645	0.895	0.718	0.018	2.735
8	3.500	1.855		0.753	0.053	1.252
9	—	—		0.788	0.088	1.526
11	3.500	1.855		0.893	0.193	0.090
13	—	—		0.998	0.298	0.756
15				1.050	0.350	

Table 28. continued.

Time of incubation	IX		
	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.	
0	0.823		
2	1.033	0.210	1.466
4	1.436	0.613	0.290
6	1.646	0.823	0.894
8	1.751	0.928	
9	1.751	0.928	

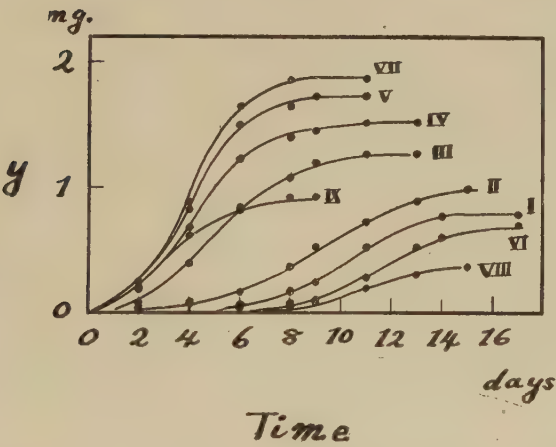


Fig. 15. Bacterial decomposition of the mixture of two kinds of meat extracts

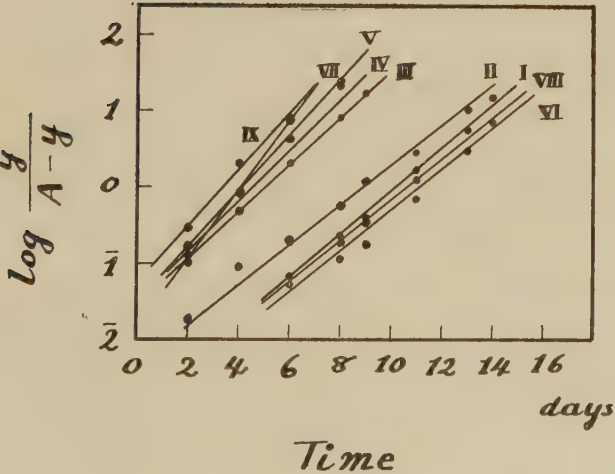


Fig. 16. Relation between $\log \frac{y}{A-y}$ and time.

fore, the equation $\log \frac{y}{A-y} = K(t-t_1)$ can be applied to the process. K and t_1 calculated from the Fig. 16 are shown in Table 30.

Table 29. Growth of bacteria.

	0 h	24 hs.
I	724	312×10^6
II		371×10^6
III		387×10^6
IV		346×10^6
V		302×10^6
VI		318×10^6
VII		278×10^6
VIII		297×10^6
IX		252×10^6

Table 30. Values of various constants.

No of flask	Total N	a	A	A/a	K	t_1
	mg.	mg.	mg.			d.
I	8.795	7.371	0.823	0.111	0.283	10.20
II	9.125	7.664	0.980	0.128	0.263	8.90
III	9.675	8.153	1.260	0.155	0.307	5.10
IV	10.225	8.642	1.523	0.176	0.323	4.60
V	10.555	8.935	1.715	0.192	0.367	4.20
VI	8.575	7.175	0.700	0.097	0.266	11.20
VII	10.775	9.130	1.855	0.203	0.450	4.20
VIII	4.288	3.588	0.350	0.097	0.266	10.70
IX	5.388	4.565	0.928	0.203	0.375	3.50

The value of K of the mixtures of meat extracts falls between those of each two components. Also, the value of t_1 is between each of the two, but it may be divided into two groups, because it is very close to either one of the values of t_1 of the meat extract contained in excess in the mixtures.

In the case of the mixtures in various concentrations of two kinds of meat extracts, the value of A/a also falls between those of each of the two. Also, the value of A for the mixtures is equal to the sum of those for both the components (Table 31). This relation is shown in Fig. 17.

The value of a for the mixture must be equal to the sum of a 's

for both components, a' and a'' , that is $a = a' + a''$. a' and a'' are determined by the chemical analysis. The value of A/a for chub mackerel is 0.203, and that for halibut is 0.097. Therefore, if a is a' in the case of chub mackerel, and if it is a'' in the case of halibut, $a' \times 0.203 = A'$ and $a'' \times 0.097 = A''$. Then, the sum of A' and A'' are calculated and denoted by A''' . It is understood that A''' is nearly equal to A of the mixture obtained by the experiment, that is

$$A' + A'' = A''' = A$$

From the above mentioned facts, it is presumed that the difference in the progression of the bacterial decomposition of meat extract of two kinds of aquatic animals such as red meat fish (chub mackerel) and white meat fish (halibut) depends on the chemical composition of the extractive matter which varies with the kind of animals, and that the unknown substance is not present in the meat-extracts, which accelerates or retards the bacterial decomposition.

Table 31.

No. of flask	a'	a''	a	A'	A''	A'''	A
	mg.	mg.	mg.	mg.	mg.	mg.	mg.
I	0.913	6.458	7.371	0.185	0.626	0.811	0.823
II	2.283	5.381	7.664	0.463	0.522	0.985	0.980
III	4.565	3.588	8.153	0.927	0.348	1.275	1.260
IV	6.848	1.794	8.642	1.390	0.174	1.564	1.523
V	8.217	0.718	8.935	1.668	0.070	1.738	1.715

The growth of bacteria in the mixtures of various concentrations of the solution of two kinds of meat extracts is nearly the same as shown in Table 29. This fact is evidently understood, from the previous experiment, that the growth of bacteria in the solution of meat extract of various kinds of aquatic animals is almost unrelated with the kinds of animals.

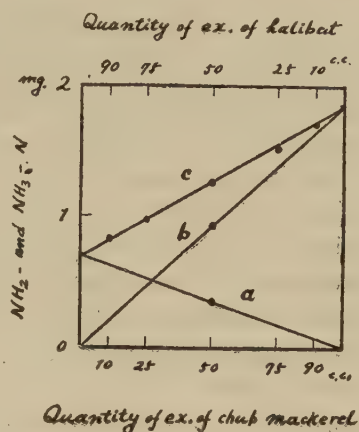


Fig. 17. Final rate of the bacterial decomposition of the mixtures in various concentrations of two kinds of meat extracts.

- a.....halibut only.
- b.....chub mackerel only.
- c.....mixture.

VI. Bacterial Decomposition of Meat Extract of Differently Aged Aquatic Animals.

So far, little is known of the relation of the age of an aquatic animal to the bacterial decomposition of it. According to HUNTER,^(56, 57) in actively feeding salmon, enzymic as well as bacterial decomposition may take place when dead. It has been found that immature salmon caught on the feeding ground, with alimentary tract full of feed, is decomposed in a manner different from that in the mature salmon caught on the spawning migration after the fish have ceased feeding, and when the alimentary tract is empty. In the immature feeding salmon enzymic decomposition is accompanied by bacterial spoilage, causing a softening of belly wall and disintegration of the viscera of the fish. In mature salmon caught on the spawning migration, the alimentary tract is sterile since no food is present and invasion by bacteria in such fish is solely from the outside. After the salmon have reached the spawning grounds, the type of the spoilage again differs from that of the mature migratory salmon even though the bacterial flora in both are almost identical. The chemical composition of the meat may vary with time in the life-cycle of animal. Therefore, it seems that the rate and the velocity of the bacterial decomposition depend also upon the time in the life-cycle of the animal at which it is caught.

In the present experiment, it was studied the types of the bacterial decomposition of meat extract of some aquatic animals of different ages, and the growth of bacteria in them.

Experiment.

The solutions of meat extract of chub mackerel, tunny, sea bream and carp of different ages were made by the previously described method, and were used in the present experiment.

One hundred cubic centimeters of these solutions in the flasks of the capacity of 200 c.c. were decomposed by *Pseudomonas fluorescens* as previously experimented, and amino and ammonium nitrogen in 5 c.c. and the number of bacteria in 1 c.c. were determined at definite intervals.

In the case of carp, age was estimated by the annular rings on the scale, but in the case of the others it was standardized by the body weight.

By the previous experiment it is known that the values of K and A/a are almost indifferent to the concentration of meat extract. Ac-

cordingly, the meat extract's solutions of one and two years old carp were diluted to 1.5 and 2.0 times with distilled water, for the samples were too little in amount. Besides, in the case of tunny, both of the original solution and the solution diluted to 2 times were used for caution's sake.

Results and Discussion.

Results obtained are given in Tables 32-35 and 37. From these data Figs. 18-21 are obtained. The value of K calculated from these figures and the value of A/a calculated from Tables 32-35 are shown in Table 36.

Table 32. Tunny.

Original solution

Time of incubation	Body weight 112.800 kg.			Body weight 2.850 kg.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.750			1.890		
2	1.838	0.088	2.504	2.188	0.298	2.905
3	—	—	—	3.063	1.173	1.691
4	1.995	0.245	2.978	4.375	2.485	0.360
5	2.275	0.525	1.357	5.215	3.325	1.133
6	3.150	1.400	1.991	5.425	3.535	2.005
7	4.113	2.363	0.701	5.460	3.570	
9	4.585	2.835		—	—	

Table 32. continued.

Diluted solution (to 2 times)

Time of incubation	Body weight 112.800 kg.			Body weight 2.850 kg.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.875			0.945	0	
2	0.963	0.088	2.799	1.400	0.455	1.512
3	1.068	0.193	1.174	2.100	1.155	0.218
4	1.330	0.455	1.644	2.625	1.680	0.983
5	1.855	0.980	0.238	2.800	1.855	
6	2.205	1.330	0.926	2.800	1.855	
7	2.275	1.400	1.203	2.800	1.855	
9	2.363	1.488		2.800	1.855	

Table 33. Chub mackerel.

Time of incubation	Body weight 1.140 kg.			Body weight 0.025 kg.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.435			1.575		
2	1.750	0.315	1.256	2.048	0.473	1.570
3	2.100	0.665	1.678	2.800	1.225	0.368
4	2.800	1.365	0.290	3.150	1.575	0.955
5	3.238	1.803	0.848	3.273	1.698	1.514
6	3.413	1.978	1.357	3.325	1.750	
7	3.500	2.065		3.325	1.750	

Table 34. Carp.

Time of incubation	6 years old			4 years old		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	1.400			1.330		
2	1.663	0.263	1.438	1.488	0.158	1.144
3	1.768	0.368	1.633	1.610	0.280	1.441
4	1.820	0.420	1.718	1.750	0.420	1.672
5	1.820	0.420	1.718	1.820	0.490	1.786
7	2.135	0.735	0.176	2.135	0.805	0.216
9	2.310	0.910	0.462	2.293	0.963	0.463
11	2.450	1.050	0.779	2.398	1.068	0.673
14	—	—	—	2.625	1.295	
17	2.625	1.225		2.625	1.295	

Table 34. continued.

Time of incubation	3 years old			2 years old			1 year old		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	1.120			0.770			0.613		
2	1.260	0.140	1.260	0.910	0.140	1.347	0.736	0.123	1.329
3	1.400	0.280	1.648	1.015	0.245	1.670	0.946	0.330	1.958
4	1.470	0.350	1.797	1.126	0.350	1.921	1.016	0.403	0.134
5	1.575	0.455	0.000	1.208	0.438	0.121	1.103	0.490	0.369
7	1.768	0.648	0.394	1.313	0.543	0.380	1.203	0.595	0.754
9	1.925	0.805	0.885	1.418	0.648	0.726	1.261	0.648	1.096
11	1.960	0.840	1.080	1.470	0.700	1.000	1.296	0.683	—
14	1.995	0.875	1.398	1.505	0.735	1.323	1.296	0.683	—
17	2.030	0.910		1.540	0.770		1.313	0.700	

Table 35. Sea bream.

Time of incubation	Body weight 4.410 kg.			Body weight 1.210 kg.			Body weight 0.330 kg.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.910			0.945			1.260		
3	0.910	0		0.945	0		1.295	0.035	2.796
5	0.910	0		0.945	0		1.400	0.140	1.489
7	0.980	0.070	1.302	1.015	0.070	1.348	1.575	0.315	0.052
9	1.015	0.105	1.524	1.120	0.175	1.922	1.750	0.490	0.670
11	1.155	0.245	0.147	1.208	0.263	0.334	1.803	0.543	1.020
13	1.260	0.350	0.699	1.260	0.315	0.654	—	—	—
15	1.295	0.385	1.042	1.295	0.350	1.000	—	—	—
18	1.330	0.420		1.330	0.385		1.855	0.595	

Table 36. Values of various constants.

Kind of animal			a	A	A/a	K	Total N
Tunny	Body weight 112.800 kg. (orig.)		mg. 9.800	mg. 2.835	0.289	0.560	mg. 11.550
	Body weight 112.800 kg. (dil.)		4.900	1.488	0.304	0.560	5.775
	Body weight 2.850 kg. (orig.)		10.405	3.570	0.343	0.750	12.295
	Body weight 2.850 kg. (dil.)		5.203	1.855	0.357	0.750	6.148
Chub mackerel	Body weight 1.140 kg.		10.290	2.065	0.201	0.540	11.725
	Body weight 0.025 kg.		8.925	1.750	0.196	0.645	10.500
Sea bream	Body weight 4.410 kg.		5.740	0.420	0.073	0.230	6.650
	Body weight 1.210 kg.		5.618	0.385	0.069	0.230	6.563
	Body weight 0.330 kg.		6.615	0.595	0.090	0.310	7.875
Carp	6 years old		6.475	1.225	0.189	0.160	7.875
	4 years old		6.808	1.290	0.190	0.160	8.138
	3 years old		5.355	0.910	0.170	0.160	6.475
	2 years old		3.605	0.770	0.214	0.160	4.375
	1 year old		3.150	0.700	0.222	0.200	3.850

K of the young fishes is greater than that of older fishes. However, it seems that *K* is nearly the same for the fishes above a certain definite age as recognized in the cases of sea bream and carp, that is, the speed of bacterial decomposition of meat extract decreases as the age of the animal advances, but becomes nearly constant above a certain definite age. Also, the value of *A/a* has a tendency similar to the value of *K*. But, in the case of chub mackerel *K* of the younger is

greater than that of the older, although A/a is nearly indifferent to age.

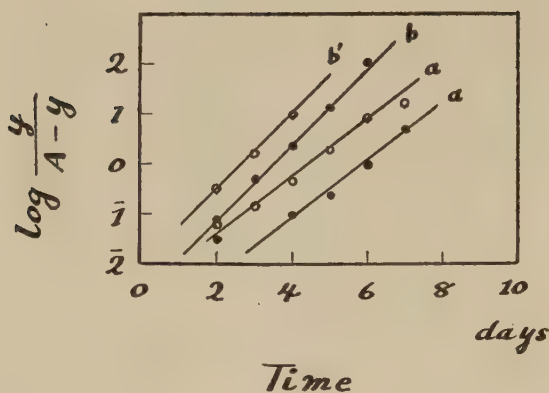


Fig. 18. Relation between $\log \frac{y}{A-y}$ and time (tunny).

a.....body weight, 112.800 kg.
 a' body weight, 112.800 kg.
 (diluted to 2 times.)
 b.....body weight, 2.850 kg.
 b' body weight, 2.850 kg.
 (diluted to 2 times.)

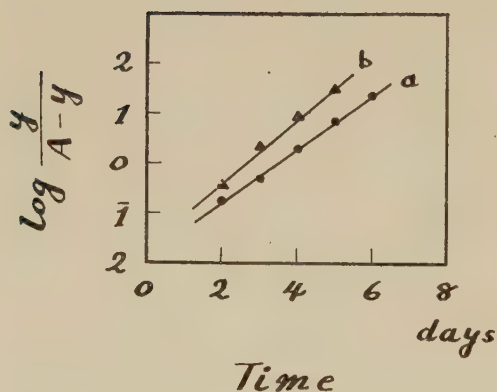


Fig. 19. Relation between $\log \frac{y}{A-y}$ and time (chub mackerel).

a.....body weight, 1.140 kg.
 b.....body weight, 0.025 kg.

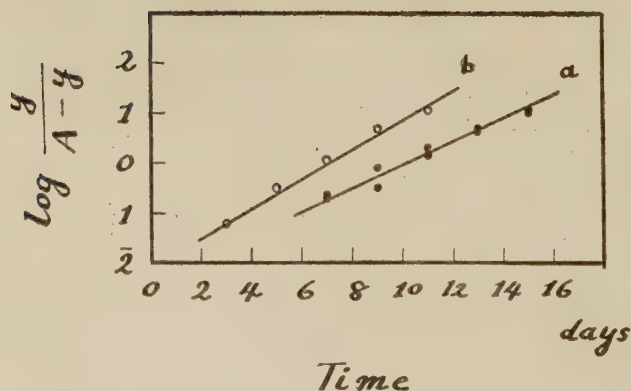


Fig. 20. Relation between $\log \frac{y}{A-y}$ and time (sea bream).

a.....body weight, 1.210 kg. and 4.410 kg.
 b.....body weight, 0.330 kg.

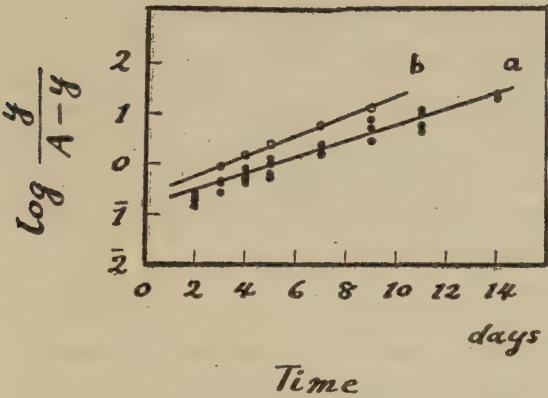


Fig. 21. Relation between $\log \frac{y}{A-y}$ and time (carp).
a.....2, 3, 4 and 6 years old.
b.....1 year old.

As the extractive matter of meat is supposed to play an important rôle on the type of the bacterial decomposition of meat of an aquatic animal, it is presumable that the similar interrelation exist between the type of the bacterial decomposition of meat and the age of the animal.

That growth of bacteria in the solution of extractive matter is, as already described, almost indifferent to the kind of aquatic animals, is again proved in the present experiment, and this is almost independent of the difference in age of the animal (Table 37).

Table 37. Growth of bacteria.

Kind of animal		0 h.	22 hs.	48 hs.
Tunny	Body weight 112.800 kg. (orig.)	1012	987 × 10 ⁵	†584 × 10 ⁶
	Body weight 112.800 kg. (dil.)		124 × 10 ⁶	258 × 10 ⁷
	Body weight 2.850 kg. (orig.)		*318 × 10 ⁶	248 × 10 ⁷
	Body weight 2.850 kg. (dil.)		*501 × 10 ⁶	236 × 10 ⁷
Chub mackerel	Body weight 1.140 kg.	281	*363 × 10 ⁶	368 × 10 ⁷
	Body weight 0.025 kg.		*492 × 10 ⁶	372 × 10 ⁷
Sea bream	Body weight 4.410 kg.	642	566 × 10 ⁵	197 × 10 ⁷
	Body weight 1.210 kg.		898 × 10 ⁵	115 × 10 ⁷
	Body weight 0.330 kg.		862 × 10 ⁵	202 × 10 ⁷
Carp	6 years old	864	913 × 10 ⁵	104 × 10 ⁷
	4 years old		615 × 10 ⁵	103 × 10 ⁷
	3 years old		121 × 10 ⁶	138 × 10 ⁷
	2 years old		127 × 10 ⁶	168 × 10 ⁷
	1 year old		145 × 10 ⁶	213 × 10 ⁷

*.....24 hours. †.....44 hours.

VII. Bacterial Decomposition of Muscle Protein of Several Kinds of Aquatic Animals.

The bacterial decomposition of meat extract of aquatic animals is almost elucidated already by the preceding experiment. But, so far as the present author is aware, the bacterial decomposition of muscle protein of aquatic animals has not yet been studied. In the present experiment he concerns with this problem.

According to BAINBRIDGE,⁽⁸⁾ RETTGER and BERMAN,⁽⁹⁰⁾ and SPERRY and RETTGER,⁽¹²⁰⁾ various bacteria are unable to initiate decomposition of purified proteins when these only constitute the nitrogen source in the medium, but, if the medium contains peptone or some similar available nitrogen source many bacteria during their growth will produce proteolytic enzymes. In the preliminary experiment it was confirmed that the bacteria (*Pseudomonas fluorescens*) do not attack the muscle protein of chub mackerel suspended in the physiological salt solution for several days, but addition of small quantity of the broth to it made the growth of bacteria as well as the decomposition of muscle protein occur.

Therefore, in the present experiment, to the muscle protein which was suspended in physiological salt solution, small quantity of the broth or meat extract of some aquatic animals was added.

Experiment.

Suspensions of muscle proteins employed were prepared from bonito, chub mackerel, halibut, serranid, sea bream and spiny lobster by the previously described method.

Fifty cubic centimeters of these suspensions were put into a sterilized flask of the capacity of 200 c.c. and mixed with 30 c.c. of the physiological salt solution and 20 c.c. of the broth, or the meat extract's solution of bonito or sea bream, and sterilized. Total nitrogen in 5 c.c. of the broth and of the meat extract's solution of bonito and sea bream are as 10.763 mg., 11.157 mg. and 6.263 mg. respectively.

To the above flask 0.1 c.c. of the bacterial suspension of *Pseudomonas fluorescens* was added, which was broth culture diluted to 1,000 times with sterilized distilled water, and was incubated at 26°C–27°C. At definite intervals, amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. were determined.

Results and Discussion.

Results obtained are shown in Tables 38-40 and 44. The relation between the production of amino and ammonium nitrogen and the time obtained from these data is shown in Figs. 22-24. The values of K and t_1 calculated from Figs. 22-24 and the value of A/a calculated from Tables 38-40 are given in Tables 41-43.

Table 38. Decomposition of proteins (in the case of addition of broth).

Time of incubation	Chub mackerel			Bonito			Sea bream		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.395			0.414			0.432		
3	0.818	0.423	2.944	0.978	0.564	1.031	0.770	0.338	2.936
5	1.730	1.335	1.534	2.069	1.655	1.604	1.278	0.846	1.394
7	2.717	2.332	1.901	3.259	2.845	1.992	1.974	1.542	1.752
9	3.855	3.460	0.288	4.231	3.817	0.286	2.820	2.388	0.104
10	4.419	4.024	0.522	4.795	4.381	0.494	3.177	2.745	0.257
12	5.077	4.682	0.919	5.555	5.141	0.899	3.760	3.328	0.550
14	5.359	4.964	1.246	5.917	5.503	1.284	4.324	3.892	1.016
16	5.547	5.152	1.740	6.205	5.791		4.512	4.080	1.337
19	5.641	5.246		6.205	5.791		4.700	4.268	

Table 38. continued.

Time of incubation	Halibut			Serranid			Spiny lobster			Bonito (dark muscle)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.		mg.	mg.	
0	0.414			0.432			0.489			0.414		
3	0.677	0.263	2.906	0.752	0.320	2.870	1.034	0.545	1.141	1.147	0.733	1.351
5	1.072	0.658	1.360	1.353	0.921	1.394	1.768	1.279	1.600	2.294	1.880	1.946
7	1.505	1.091	1.650	2.162	1.730	1.775	2.726	2.237	1.998	3.197	2.783	0.396
9	2.351	1.937	0.084	3.102	2.670	0.131	3.855	3.366	0.476	3.949	3.535	0.877
10	2.501	2.087	0.161	3.572	3.140	0.320	4.234	3.745	0.700	4.231	3.817	1.308
12	3.063	2.649	0.477	4.324	3.892	0.714	4.701	4.212	1.175	4.326	3.912	1.674
16	3.573	3.159	0.926	4.700	4.268	1.055	4.889	4.400	1.671	4.419	4.005	
19	3.761	3.347	1.252	4.888	4.456	1.376	4.983	4.494		4.419	4.005	
21	3.949	3.535		5.076	4.644		4.983	4.494		4.419	4.005	

Table 39. Decomposition of proteins
(in the case of addition of meat extract of sea bream).

Time of incubation	Chub mackerel			Sea bream			Halibut		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.315			0.309			0.309		
3	0.559	0.244	2.792	0.467	0.158	2.677	0.467	0.158	2.724
5	1.443	1.128	1.567	0.723	0.414	1.130	0.723	0.414	1.184
7	2.437	2.122	0.012	1.644	1.335	1.793	1.361	1.052	1.703
8	3.005	2.690	0.237	2.259	1.950	0.103	1.849	1.540	1.984
10	3.580	3.265	0.548	2.929	2.620	0.479	2.414	2.105	0.309
12	4.075	3.760	0.942	3.244	2.935	0.724	2.884	2.575	0.659
14	4.230	3.915	1.153	—	—	—	3.264	2.955	1.204
16	4.425	4.110	1.712	3.690	3.381	1.492	3.354	3.045	1.507
18	4.505	4.190		3.799	3.490		3.449	3.140	
21	4.505	4.190		3.799	3.490		3.449	3.140	

Table 39. continued.

Time of incubation	Spiny lobster			Bonito (dark muscle)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.376			0.282		
3	0.640	0.264	2.883	0.601	0.319	1.132
5	1.281	0.905	1.508	1.280	0.998	1.764
7	2.256	1.880	0.009	2.047	1.765	0.291
8	2.691	2.315	0.216	2.332	2.050	0.520
10	3.611	3.235	0.836	2.802	2.520	1.226
12	3.896	3.520	1.240	2.877	2.595	1.540
14	4.006	3.630	1.592	2.952	2.670	
16	4.099	3.723		2.952	2.670	
18	4.099	3.723		2.952	2.670	
21	4.099	3.723		—	—	

Both K and t_1 for the same protein depend on the kind of material added to the suspension of muscle protein, when the meat extract of bonito is added, K is larger and t_1 is smaller than in the case where the broth or the meat extract of sea bream is added. When the same kind of the material is added to the suspension of muscle proteins, the value of K for muscle proteins of spiny lobster and dark muscle

Table 40. Decomposition of proteins
(in the case of addition of meat extract of bonito).

Time of incubation	Chub mackerel			Bonito			Halibut		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.640			0.552			0.552		
3	1.543	0.903	1.385	1.455	0.903	1.348	1.717	1.165	1.600
5	3.382	2.745	0.165	3.072	2.520	0.016	2.997	2.445	0.173
7	4.420	3.780	0.652	4.312	3.760	0.499	3.972	3.420	0.703
9	4.800	4.160	0.952	5.212	4.660	1.200	—	—	—
10	5.070	4.430	1.357	5.327	4.775	1.424	4.457	3.905	1.302
12	5.220	4.580	2.007	5.507	4.955		4.652	4.100	
14	5.265	4.625		5.507	4.955		4.652	4.100	
16	5.265	4.625		5.507	4.955		4.652	4.100	
17	5.265	4.625		—	—		—		

Table 40. continued.

Time of incubation	Spiny lobster			Bonito (dark muscle)		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.694			0.552		
3	2.119	1.425	1.714	1.530	0.978	1.614
5	3.734	3.040	0.429	2.792	2.240	0.306
7	4.529	3.835	1.054	3.522	2.970	0.897
9	4.754	4.060	1.552	3.827	3.275	1.658
10	4.868	4.174		3.899	3.347	
12	4.868	4.174		3.899	3.347	
16	4.868	4.174		3.899	3.347	

(bonito) is larger than that in all other cases which latter give nearly the same value. The value of A/a of muscle protein of spiny lobster is the greatest, that of the dark muscle (bonito) is the smallest, and that of the others is nearly the same and intermediate between the two extremes.

From these facts it is evident that in the case of fish proteins, both the velocity and the final rate of the bacterial decomposition are almost indifferent to the kind of fishes, that is, either for white meat fish (such as halibut, sea bream, etc.) or for red meat fish (such as bonito, chub mackerel, etc.) both K (speed) and A/a (final rate) are nearly

identical. However, the protein of dark muscle (bonito) is different from those of the others, the value of K being larger while the value of A/a being smaller than those of the others.

The growth of bacteria is almost the same in any of the case of the suspensions of muscle protein, as shown in Table 44.

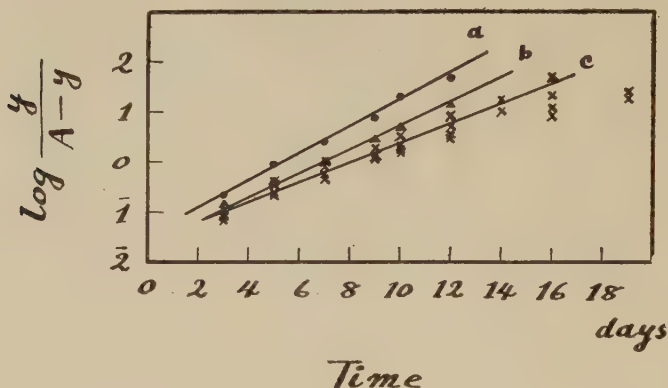


Fig. 22. Relation between $\log \frac{y}{A-y}$ and time (in the case of addition of broth).

a.....dark muscle (bonito).

b.....spiny lobster.

c.....chub mackerel, sea bream, halibut and serranid.

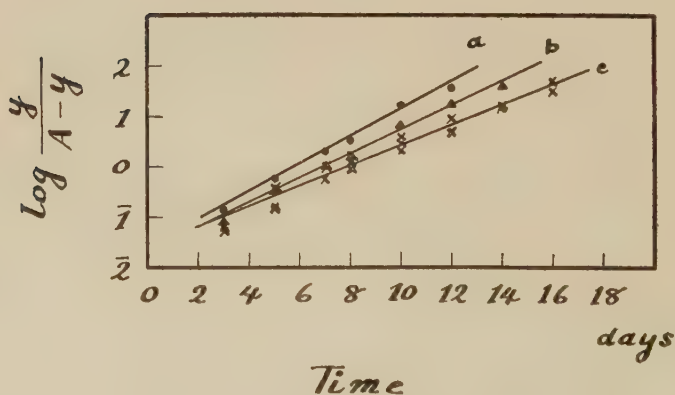


Fig. 23. Relation between $\log \frac{y}{A-y}$ and time (in the case of addition of meat extract of sea bream).

a, b and c are the same in Fig. 22

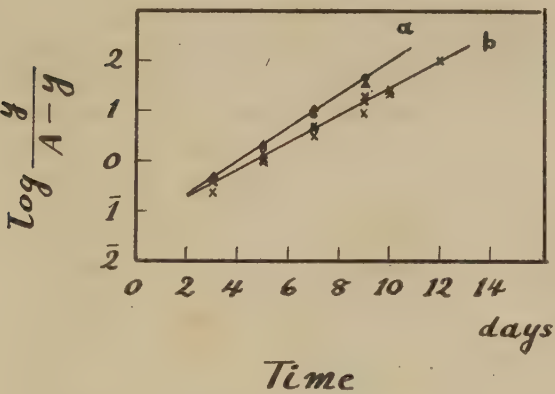


Fig. 24. Relation between $\log \frac{y}{A-y}$ and time (in the case of addition of meat extract of bonito).
a.....spiny lobster and dark muscle.
b.....chub mackerel, bonito and halibut.

Table 41. Values of various constants (in the case of addition of broth).

Proteins	Total N	a	A	A/a	K	t ₁
	mg.	mg.	mg.			d.
Chub mackerel	15.454	15.059	5.246	0.349	0.200	8.10
Bonito	16.954	16.540	5.791	0.349	0.200	8.10
Sea bream	12.741	12.309	4.268	0.346	0.200	8.10
Halibut	11.592	11.178	3.535	0.325	0.200	8.10
Serranid	14.217	13.785	4.644	0.337	0.200	8.10
Spiny lobster	9.604	9.115	4.494	0.492	0.240	6.90
Bonito (dark muscle)	15.529	15.115	4.005	0.265	0.265	5.35

Table 42. Values of various constants (in the case of addition of meat extract of bonito).

Proteins	Total N	a	A	A/a	K	t ₁
	mg.	mg.	mg.			d.
Chub mackerel	15.573	14.933	4.625	0.310	0.265	4.70
Bonito	17.022	16.470	4.955	0.300	0.265	4.70
Halibut	12.402	11.850	4.100	0.346	0.265	4.70
Spiny lobster	9.739	9.045	4.174	0.462	0.330	4.00
Bonito (dark muscle)	15.597	15.045	3.347	0.222	0.330	4.00

Table 43. Values of various constants (in the case of addition of meat extract of sea bream).

Proteins	Total N	a	A	A/a	K	t ₁
	mg.	mg.	mg.			d.
Chub mackerel	14.099	13.784	4.190	0.304	0.200	7.90
Sea bream	10.943	10.634	3.490	0.328	0.200	7.90
Halibut	10.213	9.904	3.140	0.317	0.200	7.90
Spiny lobster	8.217	7.841	3.723	0.476	0.240	7.00
Bonito (dark muscle)	14.161	13.879	2.670	0.192	0.275	5.80

Table 44. Growth of bacteria in the suspensions of muscle protein.
(in the case of addition of broth)

Kind	0 h.	22 hs.	48 hs.	72 hs.
Chub mackerel	} 308	238×10^5	589×10^6	164×10^7
Bonito		258×10^5	502×10^6	128×10^7
Sea bream		188×10^5	823×10^6	137×10^7
Halibut		301×10^5	499×10^6	123×10^7
Serranid		235×10^5	433×10^6	121×10^7
Spiny lobster		283×10^5	489×10^6	136×10^7
Bonito (dark muscle)		233×10^5	421×10^6	132×10^7

(in the case of addition of meat extract of sea bream)

Chub mackerel	} 289	311×10^5	523×10^6	799×10^6
Sea bream		208×10^5	518×10^6	102×10^7
Halibut		215×10^5	383×10^6	793×10^6
Spiny lobster		182×10^5	499×10^6	863×10^6
Bonito (dark muscle)		253×10^5	428×10^6	735×10^6

(in the case of addition of meat extract of bonito)

Chub mackerel	} 322	$*758 \times 10^5$	—	118×10^7
Bonito		$*528 \times 10^5$	—	982×10^6
Halibut		$*871 \times 10^5$	—	138×10^7
Spiny lobster		$*667 \times 10^5$	—	145×10^7
Bonito (dark muscle)		$*832 \times 10^5$	—	126×10^7

*.....24 hours.

VIII. Summary.

Under the suitable condition, the time required by bacteria for reaching the maximum number is nearly the same irrespective of their initial amounts, and again when the bacterial cells reach the maximum, the number is nearly the same, so that, the generation time of bacteria depends on the number of the initial inoculated cells and becomes longer with larger amount of the inoculum.

The bacterial decomposition is rapidly increased, after the bacterial cells reached the maximum in number. From these facts it is evident that the meat of aquatic animals is similarly decomposed irrespective of the initial number of bacteria already present.

The progress of the bacterial decomposition of muscles, meat extracts and muscle proteins of aquatic animals can be defined equally by the equation

$$\log \frac{y}{A - y} = K(t - t_1)$$

which is derived from the equation expressing the speed of a monomolecular autocatalytic chemical reaction:

$$\frac{dy}{dt} = kt(a - y)$$

In the equation there are two variables: y , the metabolic product, and t , the time, and three constants: K , A and t_1 . The constant K is significant of the kind of materials which are decomposed, that is, in the case of the same material it is nearly the same. Therefore, the speed of the bacterial decomposition is capable of being compared by the value of K . When the speed of the decomposition of meat extracts is compared by the value of K , red meat fish (such as bonito, chub mackerel, etc.) has the greatest, white meat fish (such as serranid, sea bream, halibut, etc.) has the smallest and carangoid has the intermediate value, while K for crustacea (such as spiny lobster) is greater than that for all three groups of fishes. In the case of muscle proteins, K 's of crustacea and dark muscle of fish are greater than the other fishes which latter are nearly the same. From these facts, it may be presumed that the velocity of the bacterial decomposition of meat of crustacea is great, because those of both the extractive matter and the muscle protein are great, and that K of the meat of fishes is mainly dependent upon that of the extractive matter and is larger for those of the extractive matter having larger K , because K of any one of the muscle proteins of fishes is almost indifferent.

In the case of animals of the same kind, the value of K of meat extract is nearly the same for fishes above a certain age, but below that age it decreases as age of the animal advances. Therefore, it may be presumed that the muscle of younger animal is more easily decomposed by bacteria than that of older, but above a certain definite age, it is nearly indifferent to age.

The constant A , representing the total amount of the metabolic product, must be influenced by increasing or decreasing concentration of the original substrate, because it is a fractional part of a , representing the original concentration of the substrate. A/a is independent of the original concentration for the same kind of the substrate, provided the original concentration of the substrate is not extraordinarily dilute. Therefore, the final rate of the bacterial decomposition can be compared by the value of A/a . The value of A/a has the same tendency

as the value of K , namely, when the velocity of the decomposition is large, the rate of the decomposition is also large. But the muscle protein of dark muscle forms exception to all of the others, i.e. although the value of K is large, the value of A/a is small.

The constant t_1 , representing the time at which the reaction is half completed, i.e. $y = \frac{A}{2}$, depends on the original concentration for the same kind of the substrate, and becomes larger with increasing concentration. In other words, the period in which the decomposition is rapidly in progress, i.e. inductive period (or lag period) becomes longer with increasing concentration.

In the case of the mixture of two kinds of meat extract, any of the three constants K , A/a and t_1 falls between the values for the two components of the mixture and they are close to those of component contained much in the mixture.

The difference in the type of the bacterial decomposition of meat extract depends on the chemical composition of the extractive matter, which varies with the kind of animals, and the unknown substance is not present in the meat extract, which accelerates or inhibits the bacterial decomposition.

The growth of bacteria in the solution of meat extract or in the muscle suspension is independent of the kind of animals, if the original concentration of the substrate is not very dilute. Therefore, it is understood that the bacterial growth is similar, although the bacterial decomposition varies with the kind of animals.

PART THREE

Relation between Autolytic and Bacterial Agencies in the Decomposition of Muscle of Some Aquatic Animals.

As already described, purified proteins are unable to be attacked by bacteria and also proteoses and higher polypeptides are resistant to the direct attack of organisms and if the medium contains peptone or some similar available nitrogen source many bacteria will produce during their growth proteolytic enzymes. In the previous experiment, the present author has confirmed that bacteria can not attack the muscle protein of fish which is suspended in physiological salt solution, but if the medium contains small quantity of meat extract, bacteria can initiate both their growth and the decomposition of protein. ROBINSON and RETTGER⁽⁹⁰⁾ found that bacteria do not require proteins, even in minute quantities, to develop normally, but obtain their substances from the amino acid and perhaps some of the simpler polypeptides. Therefore, in the case of bacterial decomposition of meats, it may be presumed that at first bacteria grow, utilizing the extractive matter in the meat, and then they become able to initiate decomposition of muscle protein, after they have grown to a certain extent.

GRUCKSHANK⁽⁴³⁾ investigating the histological change in autolyzing kidney found that *Staph. aureus* introduced into kidney which had autolyzed 24 hours at 37°C, produced very little change during the first day, although there was active multiplication. During the next 24 hours, however, there was a rapid softening and cellular change. On the other hand, these organisms introduced into fresh kidney produced a complete necrosis in 10 to 15 hours, as compared to 12 to 15 days required by autolytic enzymes. This may be explained by the fact that the organisms first use up the simpler products of autolysis before attacking the more complex substances. RICE⁽⁹¹⁾ found this to be the case when organisms were introduced into clam muscle previously autolyzed in toluol and then autoclaved to sterilize and drive off the toluol. At first there was a decrease in non-coagulable nitrogen, that is, the simpler products of autolysis were utilized. Therefore, it may be argued that in the meat with decreasing tissue protein and increasing soluble nitrogen by autolysis there is rapid growth of microorganisms and rapid decomposition of meat as well. If so, the more rapid bacterial decomposition of meat must be, the greater the velocity of autolysis.

It is believed that chub mackerel is more easily spoiled than sea bream. And also truly the velocity of autolysis in chub mackerel is larger than that in sea bream.⁽⁸⁰⁾

In an investigation on the rates of autolytic and bacterial decomposition of haddock, lobster and clam, REED, RICE and SINCLAIR⁽⁸⁸⁾ observed that when bacteria act along with autolytic enzymes, "contents of non-coagulable nitrogen and ammonium nitrogen increase during the first 12-24 hours only very slightly more than or not more than under the influence of the tissue enzymes alone; but from this time under the combined action of tissue enzymes and bacteria the rate of protein transformation increases enormously over that under the action of the tissue enzymes alone". Also, this fact was already recognized by other investigators. But even bacteria act alone without the influence of autolytic enzymes (i. e. when the tissue enzymes are destroyed) bacterial decomposition increases during the first period only slightly, but increases enormously after that period. Therefore, it may not be due to autolysis. In the previous experiment the author has shown that bacteria can develop normally irrespective of the concentration of nutrient which is above a certain definite value.

According to GIBBONS and REED,⁽⁸⁹⁾ the rate of growth of *Prot. vulgaris* does not seem to be influenced by the length of time autolysis has gone on before inoculation, viz., the degree of autolysis preceding the introduction of the organisms does not affect the growth rate of *Prot. vulgaris*.

So, it may be erroneous to say that simpler compounds are produced from tissue protein by autolysis prior to enormous bacterial development in the meat, none the less that bacterial growth becomes in a flourishing condition with the progress of autolysis.

Hitherto, only early stage of the decomposition has been studied in the case where bacterial growth is inhibited by an antiseptic such as toluol or what not, the action of autolytic enzymes alone going on, and also in the case where there is the combined action of autolytic enzymes and bacteria. The case of the bacterial action alone, therefore, must also be taken into consideration and, moreover, not only the early stage but also whole of the processes of the decomposition must be studied.

It is well known fact that certain animal tissues decompose more rapidly than others, but the studies, hitherto gone on, has not determined whether this is due to differences in the rate of autolysis or of bacterial activity. With these considerations in mind, the following problem was attacked.

I. Bacterial Decomposition of Muscles in Different Stages during Autolysis.

In connection with the types of bacterial decomposition and of bacterial growth, a question is raised as to whether fresh meat and autolyzed meat are practically different or not. The present study was carried out to decide the relation between the two alternatives.

Experiment.

Preparation of samples:—

Chub mackerel and sea bream, as fresh as possible were obtained.

According to OYA,⁽⁸⁰⁾ optimum hydrogen ion concentration for the autolysis of these muscles is about $\text{pH} = 4.5$, optimum temperature is about 40°C , and under such a condition, the degree of autolysis is multiplied several times or more as compared with the ordinary condition (e. g. standing after death at pH of 6.0–7.0 and at room temperature). In the present experiment, the meats were, thus, autolyzed extraordinarily, and avoided from bacterial decomposition during autolysis as follows:

Finely granulated fresh meats were freed from connective tissue by means of a strainer. Two hundred and fifty grams of these flesh pastes were mixed with 50 c.c. of N/1 HCl and filled to 1,500 c.c. with distilled water. These muscle suspensions were found to have pH values of 4.4–4.5, and 200 c.c. of them were taken into each flask of a capacity of 300 c.c., sealed with paraffine and were kept at 37°C . After standing for a definite time, each flask was taken out and neutralized with 6.66 c.c. of N/1 NaOH. At this time each flask was examined for growth of microorganisms with a microscope and by means of a culture, but bacteria were found never, although molds were found in some flasks. Flasks with mold growing were omitted. And then each flask was heated in Koch's steam sterilizer for 30 minutes. The coagulated matter was then granulated and filled to 400 c.c. with distilled water, and each quater was put into a sterilized flask of a capacity of 200 c.c. and sterilized. Nitrogen distribution in 5 c.c. of these suspensions is given in Table 45. From the data in Table 45, Figs. 25 and 26 are obtained. It is evident that autolysis of chub mackerel is enormously more progressed than that of sea bream.

Procedure:—

To each of the above flasks were added 0.1 c.c. of the bacterial suspension of *Pseudomonas fluorescens* which was broth culture diluted

Table 45. Various nitrogens during autolysis.

Kind	Days	Total N	Protein N	Soluble N	Amino N
		mg.	mg.	mg.	mg.
Chub mackerel	0	15.540	12.530	3.010	0.455
	2	15.540	6.965	8.575	1.820
	3	15.540	5.530	10.010	2.100
	5	15.540	4.690	10.850	2.450
Sea bream	0	16.100	14.210	1.890	0.385
	1	16.100	13.580	2.520	0.560
	2	16.100	12.880	3.220	0.700
	3	16.100	12.250	3.850	0.788
	4	16.100	11.900	4.200	0.868
	5	16.100	11.760	4.340	0.998

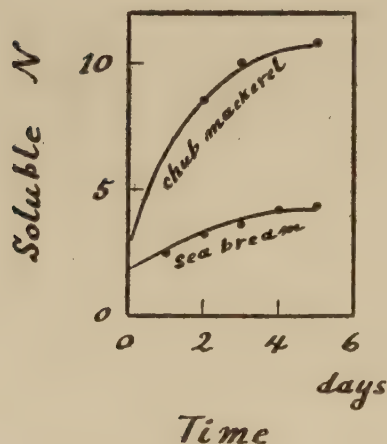


Fig. 25. Increase in soluble nitrogen during autolysis.

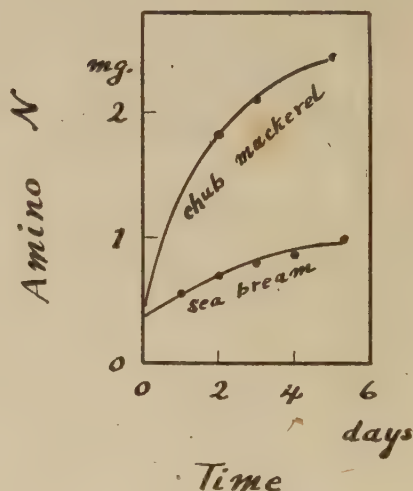


Fig. 26. Increase in amino nitrogen during autolysis.

to 1,000 times with sterilized distilled water, and was incubated at 26°C. At definite intervals, amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. of the suspension were measured.

Results and Discussion.

Results obtained are shown in Tables 46 and 47. From the data Figs. 27 and 28 are plotted. The values of K and t_i calculated from these figures are shown in Tables 48 and 49.

The value of K is almost independent of the degree of autolysis.

In other words, K has nearly the same value for fresh meat and for autolyzed meat. In the case of chub mackerel, K is slightly larger for enormously autolyzed meat than for fresh meat. But, when autolysis occurs under the ordinary condition ($\text{pH} = 6.0\text{--}7.0$, at room temperature), the production of soluble nitrogen reaches the equilibrium in lower concentration than one-tenth of that under the experimental condition.⁽⁸⁰⁾ Therefore, it follows that even when the muscle is exposed to air until autolysis reaches the equilibrium, the concentra-

Table 46. Chub-mackerel.

Time of incubation	Fresh			Autolysis during 2 days		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			1.820		
2	0.735	0.280	2.818	2.870	1.050	1.425
3	1.173	0.718	1.276	3.360	1.540	1.648
5	2.275	1.820	1.828	4.883	3.063	0.198
6	2.870	2.415	0.058	5.425	3.605	0.412
7	3.185	2.730	0.181	5.775	3.955	0.576
8	3.675	3.220	0.390	—	—	—
10	4.445	3.990	0.866	6.510	4.690	1.164
12	4.675	4.220	1.131	6.685	4.865	1.542
14	—	—	—	6.773	4.953	1.982
17	4.988	4.533		6.825	5.005	

Table 46. continued.

Time of incubation	Autolysis during 3 days			Autolysis during 5 days		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	2.100			2.450		
2	3.255	1.155	1.489	3.710	1.260	1.562
3	3.885	1.785	1.760	4.305	1.855	1.811
5	5.285	3.185	0.269	6.003	3.553	0.481
6	5.880	3.780	0.529	6.405	3.955	0.711
7	6.213	4.113	0.719	6.738	4.288	0.992
8	6.475	4.375	0.921	6.913	4.463	1.232
10	6.738	4.638	1.248	7.088	4.638	1.726
12	6.948	4.848	1.969	—	—	—
17	7.000	4.900		7.175	4.725	

Table 47. Sea bream.

Time of incubation	Fresh			Autolysis during 1 day			Autolysis during 2 days		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.385			0.560			0.700		
3	0.578	0.193	2.669	0.753	0.193	2.708	0.875	0.175	2.639
5	1.050	0.665	1.259	1.085	0.525	1.180	1.208	0.508	1.152
7	1.978	1.593	1.765	2.013	1.453	1.759	2.100	1.400	1.700
8	2.625	2.240	0.028	2.625	2.065	0.032	2.975	2.275	0.072
10	3.500	3.115	0.405	3.413	2.853	0.400	3.850	3.150	0.478
12	4.288	3.903	0.952	4.200	3.640	1.017	4.550	3.850	1.042
13	4.375	3.990	1.057	4.288	3.728	1.154	4.725	4.025	1.362
16	4.638	4.253	1.691	4.550	3.990		4.900	4.200	
20	4.725	4.340		4.550	3.990		4.900	4.200	

Table 47. continued.

Time of incubation	Autolysis during 3 days			Autolysis during 4 days			Autolysis during 5 days		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.788			0.868			0.998		
3	1.103	0.315	2.919	1.306	0.436	1.076	1.453	0.455	1.099
5	1.523	0.735	1.339	1.736	0.868	1.425	1.926	0.928	1.470
7	2.626	1.838	1.908	2.898	2.030	1.985	3.238	2.240	0.088
8	3.238	2.450	0.168	3.336	2.468	0.172	3.676	2.678	0.282
10	3.851	3.063	0.466	4.036	3.168	0.519	4.376	3.378	0.684
12	4.638	3.850	1.166	4.823	3.955	1.354	4.726	3.728	1.028
13	4.726	3.938	1.353	4.858	3.990	1.455	4.901	3.903	1.349
16	4.901	4.113		4.911	4.043	1.668	5.023	4.025	1.881
20	4.901	4.113		4.998	4.130		5.076	4.078	

Table 48. Values of various constants.
(chub mackerel)

Degree of autolysis	a	A	A/a	K	t ₁
Fresh	mg. 15.088	mg. 4.533	0.300	0.220	d. 6.10
2 days	13.720	5.005	0.364	0.220	4.30
3 days	13.440	4.900	0.364	0.220	4.30
5 days	13.090	4.725	0.361	0.250	3.10

Table 49. Values of various constants.
(sea bream)

Degree of autolysis	<i>a</i>	<i>A</i>	<i>A/a</i>	<i>K</i>	<i>t</i> ₁
Fresh	mg. 15.715	mg. 4.340	0.276	0.245	7.50
1 day	15.540	3.990	0.257		
2 days	15.400	4.200	0.273		
3 days	15.312	4.113	0.268		
4 days	15.232	4.130	0.271		
5 days	15.112	4.078	0.270		

tion of the soluble nitrogen thus formed in the muscle is less than that of soluble nitrogen produced in muscle by one-day's autolysis in the already mentioned experiment. From this fact, it appears that the initial concentration of autolytic products has little influence upon the value of *K* in the subsequent bacterial decomposition of meats.

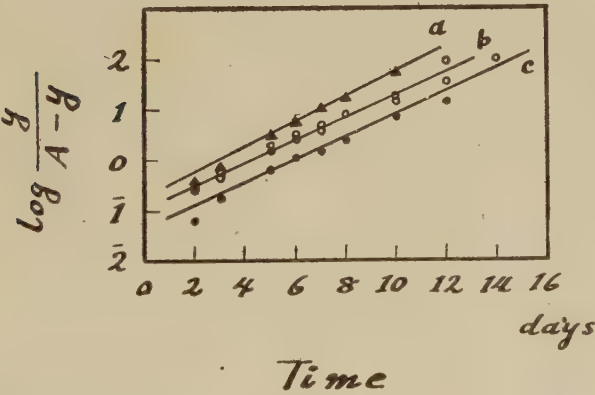


Fig. 27. Relation between $\log \frac{y}{A-y}$ and time (chub mackerel).

a.....autolysis during 5 days.
b.....autolysis during 2 and 3 days.
c.....autolysis during 0 days (fresh meat).

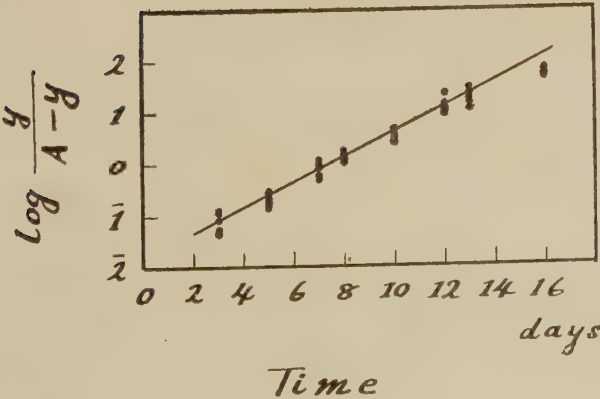


Fig. 28. Relation between $\log \frac{y}{A-y}$ and time (sea bream).

Also, A/a has a like tendency with K .

The value of t_1 is smaller for a autolyzed meat than for a fresh meat in the case of chub mackerel, but is indifferent in the case of sea bream.

From the above mentioned facts, it is evident that when the quantity of the autolytic products becomes very much, K and A/a become slightly larger and t_1 becomes smaller, but when the autolysis has advanced under the ordinary condition, K , A/a and t_1 are not influenced by the concentration of the autolytic products.

Table 50. Bacterial growth in the autolyzed meat.
(chub mackerel)

Degree of autolysis	Oh.	24 hs.	48 hs.	72 hs.
Fresh	452	482×10^5	124×10^7	198×10^7
2 days	452	473×10^5	122×10^7	207×10^7
3 days	452	562×10^5	131×10^7	212×10^7
5 days	452	508×10^5	113×10^7	217×10^7

(sea bream)

Fresh	389	469×10^5	$*628 \times 10^6$	128×10^7
1 day	389	218×10^5	$*721 \times 10^6$	116×10^7
2 days	389	423×10^5	$*579 \times 10^6$	108×10^7
3 days	389	428×10^5	$*528 \times 10^6$	123×10^7
4 days	389	598×10^5	$*693 \times 10^6$	121×10^7
5 days	389	482×10^5	$*552 \times 10^6$	118×10^7

*.....44 hours.

The growth of bacteria is almost indifferent to the concentration of the autolytic products as shown in Table 50.

II. Bacterial Decomposition of Mixture of Fresh Muscle
and Autolytically Decomposed Muscle.

The present study aims further to make sure of the results obtained in Chapter I.

By mixing fresh muscle with autolyzed muscle in various proportion, the muscle suspensions were prepared, which contain various amount of autolytic products, and their bacterial decomposition were studied.

Experiment.

Chub mackerel and sea bream as fresh as possible were used.

Finely granulated fresh meats were freed from the connective tissue contained in them by means of a strainer. Two hundred and fifty grams of these meat pastes were mixed with 1,000 c.c. of distilled water, heated in KOCH's steam sterilizer for 1 hour, finely granulated again and then were filled to 1,500 c.c. with distilled water. These suspensions were mixed with the muscle suspension autolytically decomposed for a week which had been made by the same method as given in Chapter I and with distilled water in a flask of a capacity of 200 c.c. as shown in Table 51. Nitrogen distribution in 5 c.c. of the mixtures in these flasks is given in Table 52.

Table 51. Composition of the mixtures.

Kind of fish	Chub mackerel						Sea bream			
No. of flask	I	II	III	IV	V	VI	I	II	III	IV
Autolyzed meat	0 c.c.	5	10	20	30	40	0	10	20	30
Fresh meat	60 c.c.	55	50	40	30	20	50	40	30	20
Distilled water	60 c.c.	60	60	60	60	60	50	50	50	50

Table 52. Various nitrogens of the mixtures.

Kind of fish	No. of flask	Total N	Protein N	Soluble N	Amino N
Chub mackerel	I	mg. 13.565	mg. 10.800	mg. 2.765	mg. 0.420
	II	13.455	9.920	3.535	0.788
	III	13.373	9.053	4.320	1.015
	IV	13.290	7.450	5.840	1.593
	V	13.050	5.668	7.382	2.205
	VI	12.850	3.930	8.920	2.695
Sea bream	I	15.225	13.335	1.890	0.420
	II	15.344	12.964	2.380	0.525
	III	15.463	12.593	2.870	0.665
	IV	15.582	12.222	3.360	0.770

These flasks were then sterilized, and were added to each, 0.1 c.c. of the bacterial suspension and were kept at 26°C as in Chapter I. Amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. were determined by the already mentioned method.

Results and Discussion.

Results obtained are given in Tables 53, 54 and 56, and illustrated in Figs. 29 and 30. The values of K and t_1 calculated from these figures and the value of A/a calculated from the data in Tables 53 and 54 are given in Table 55.

Table 53. Chub mackerel.

Time of incubation	I			II			III		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.420	—	—	0.788	—	—	1.015	—	—
2	—	—	—	0.963	0.175	2.658	1.295	0.280	2.880
3	0.735	0.315	2.922	1.278	0.490	1.143	1.628	0.613	1.262
4	1.278	0.858	1.424	1.873	1.085	1.568	2.363	1.348	1.711
5	1.820	1.400	1.716	2.713	1.925	1.963	3.150	2.135	0.065
7	3.325	2.905	0.388	3.938	3.150	0.557	4.200	3.185	0.607
9	4.200	3.780	1.080	4.603	3.815	1.261	4.760	3.745	1.216
11	—	—	—	4.813	4.025	—	4.900	3.885	1.645
14	4.515	4.095	—	4.813	4.025	—	4.988	3.973	—

Table 53. continued.

Time of incubation	IV			V			VI		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	1.593	—	—	2.205	—	—	2.695	—	—
2	1.978	0.385	1.006	2.818	0.613	1.235	3.465	0.770	1.435
3	2.591	0.998	1.496	3.448	1.243	1.627	4.095	1.400	1.803
4	3.326	1.733	1.846	4.550	2.345	0.114	5.075	2.380	0.289
5	4.201	2.608	0.220	5.250	3.045	0.429	5.775	3.080	0.769
7	5.251	3.658	0.843	5.950	3.745	0.932	6.213	3.518	1.606
9	5.601	4.008	1.368	6.300	4.095	1.668	—	—	—
11	5.723	4.130	1.892	6.388	4.183	—	6.300	3.605	—
14	5.776	4.183	—	6.388	4.183	—	6.300	3.605	—

The values of K , t_1 and A/a and the growth of bacteria have a tendency as in the case of Chapter I.

The values of K and A/a are almost indifferent to the amount of autolytic products excepting the case of great amount of the latter. The value of t_1 becomes smaller with increasing amount of autolytic

Table 54. Sea bream.

Time of incubation	I			II		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.420			0.525		
3	0.473	0.053	2.197	0.630	0.105	2.502
5	0.630	0.210	2.815	0.945	0.420	1.148
7	0.980	0.560	1.291	1.663	1.138	1.700
8	1.575	1.155	1.707	2.275	1.750	0.022
9	2.100	1.680	1.983	2.625	2.100	0.204
11	2.888	2.468	0.410	3.405	2.880	0.733
12	3.238	2.818	0.663	3.675	3.150	1.078
15	3.710	3.290	1.371	3.850	3.325	1.578
17	3.850	3.430		3.938	3.413	
19	3.850	3.430		3.938	3.413	

Table 54. continued.

Time of incubation	III			IV		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.665			0.770		
3	0.823	0.158	2.672	0.945	0.175	2.719
5	1.313	0.648	1.351	1.453	0.683	1.382
7	2.223	1.558	1.897	2.415	1.645	1.944
8	2.975	2.310	0.276	3.150	2.380	0.321
9	3.325	2.660	0.484	3.500	2.730	0.540
11	3.938	3.273	1.097	4.025	3.255	1.092
12	4.025	3.360	1.284	4.113	3.343	1.282
15	4.183	3.518	2.316	4.288	3.518	
17	4.200	3.535		4.288	3.518	
19	4.200	3.535		4.288	3.518	

products. The growth of bacteria is approximately the same irrespective of the amount of autolytic products.

Therefore, it is clear that the increase of autolytic products does not exert influence on the speed (K) and the final rate (A/a) of the decomposition, but shortens the inductive period of the decomposition, viz., the length of the time required for the decomposition to increase enormously, becomes shorter.

Table 55. Values of various constants.

Kind of fish	No. of flask	<i>a</i>	<i>A</i>	<i>A/a</i>	<i>K</i>	<i>t</i> ₁
Chub mackerel		mg.	mg.			d.
	I	13.145	4.095	0.311	0.310	6.10
	II	12.667	4.025	0.318	0.310	5.40
	III	12.358	3.973	0.322	0.310	4.80
	IV	11.697	4.183	0.358	0.310	4.80
	V	10.845	4.183	0.385	0.310	4.10
	VI	10.155	3.605	0.356	0.450	3.40
Sea bream	I	14.805	3.430	0.232	0.280	9.30
	II	14.819	3.413	0.230	0.280	8.25
	III	14.798	3.535	0.239	0.280	7.40
	IV	14.812	3.518	0.237	0.280	7.40

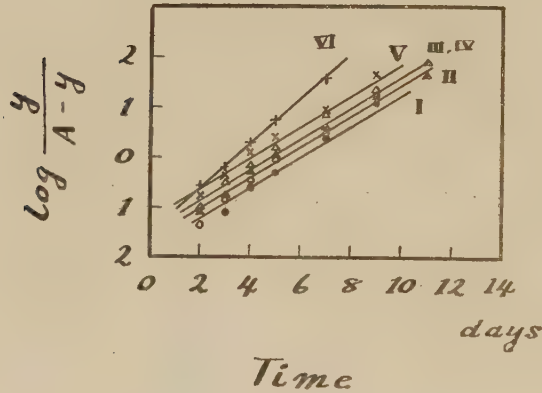


Fig. 29. Relation between $\log \frac{y}{A-y}$ and time (chub mackerel).

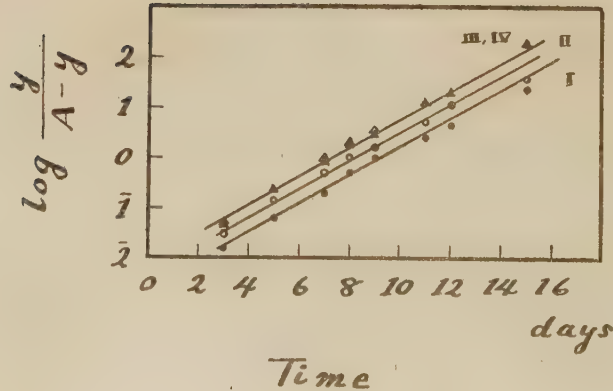


Fig. 30. Relation between $\log \frac{y}{A-y}$ and time (sea bream).

Table 56. Growth of bacteria.

Kind of fish	No. of flask	0 h.	24 hs.	48 hs.	72 hs.
Chub mackerel	I	721	408×10^5	682×10^6	189×10^7
	II		481×10^5	518×10^6	154×10^7
	III		348×10^5	673×10^6	173×10^7
	IV		768×10^5	109×10^7	184×10^7
	V		713×10^5	862×10^6	163×10^7
	VI		553×10^5	103×10^7	224×10^7
Sea bream	I	568	498×10^5	673×10^6	145×10^7
	II		489×10^5	793×10^6	152×10^7
	III		393×10^5	121×10^7	181×10^7
	IV		498×10^5	823×10^6	185×10^7

III. Bacterial Decomposition of Muscle Suspension Containing Autolytic Enzymes.

In the previous experiments, the effect of the increase of autolytic products on the bacterial decomposition of meats was ascertained.

The present study aims to examine how the effect of autolytic enzymes themselves is made on the bacterial decomposition of meats.

Generally speaking, little is known hitherto about bacterial growth or bacterial activity in the medium containing various enzymes. AOKI⁽³⁾ reported that lipase or trypsin in the medium changes bacterial growth and bacterial activity and their morphological and cultural characteristics as well.

Experiment.

In the present experiment, caution was taken to examine the decomposition under the condition as same as possible, in three cases, where there is either bacterial action alone, or action of autolytic enzymes alone and or otherwise, combined action of bacteria and autolytic enzymes. Raw meats can not be used. Therefore, it should go as the best method, to mix the heat-sterilized muscle suspension with the solution of autolytic enzymes prepared aseptically.

Materials :—

Chub mackerel, sea bream and spiny lobster were employed as fresh as possible.

Preparation of enzyme solutions :—

Fresh muscles were finely granulated, added to the same weight of glycerine, and kept at about 0°C for 24 hours, and then filtered,

The filtrates were centrifuged. These solutions were added to twice the weight of distilled water and sterilized by means of the filtration through the filter cylinder, CHAMBERLAND-PASTEUR L₂.

Preparation of muscle suspensions:—

Two hundred and fifty grams of finely granulated fresh muscles were added to 1,500 c.c. of distilled water, heated in KOCH's steam sterilizer for one hour, filtered, washed with hot distilled water for several times in order to remove soluble matters, and were finely granulated again, and then were filled to 1,500 c.c. with distilled water.

Procedure:—

Fifty cubic centimeters of the muscle suspensions were put into a flask of a capacity of 200 c.c. and sterilized and then were mixed with 30 c.c. of the enzyme solution and 20 c.c. of sterilized distilled water. Nitrogen distribution in 5 c.c. of the mixtures in the flasks is listed in Table 57.

Table 57. Nitrogens in the mixture.

Kind of fish	Total N	Protein N	Soluble N	Amino N
	mg.	mg.	mg.	mg.
Chub mackerel	18.415	15.420	2.995	0.473
Sea bream	14.586	13.368	1.218	0.280
Spiny lobster	8.806	6.405	2.401	1.120

Three flasks were thus prepared for each experimental animal. One of the three was heated in KOCH's steam sterilizer for 30 minutes, the enzymes being inactivated; and 0.1 c.c. of the bacterial suspension was added to it, and incubated at 26°C, namely the decomposition occurred by the action of bacteria alone. One of the others was not heated and 0.1 c.c. of the bacterial suspension was added to it, and kept at 26°C, namely the suspension was decomposed by both the action of autolytic enzymes and the bacterial action. The other was kept at 26°C immediately after the preparation and subjected to decomposition by the action of autolytic enzymes only. At definite intervals, amino and ammonium nitrogen in 5 c.c. and the bacterial counts in 1 c.c. of the mixtures were determined in all of the above mentioned three by the already mentioned method. The bacteriell suspension employed is the broth culture of *Pseudomonas fluorescens* diluted to 1,000 times with sterilized distilled water.

Results and Discussion.

Results obtained are listed in Tables 58-61. Figs. 31-33 are drawn from the data in these Tables.

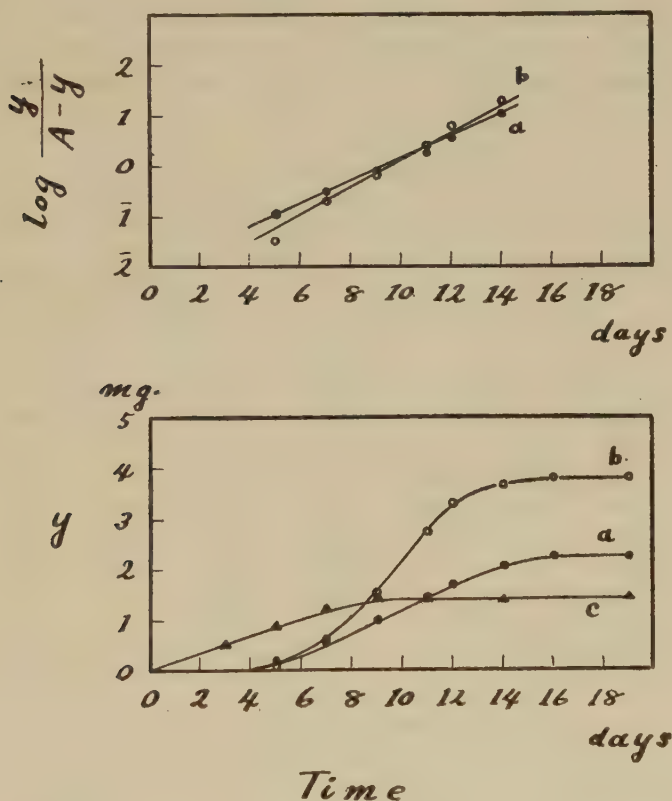


Fig. 31. Influence of autolytic enzymes on the bacterial decomposition (in the case of chub mackerel).

a.....bacterial action only.

b.....combined action of bacteria and autolytic enzymes.

c.....action of autolytic enzymes only (represented with ten times of the determining value).

It is evident that even in the case of the combined action of autolytic enzymes and bacteria the progress of the decomposition of muscles can be defined by the equation

$$\log \frac{y}{A-y} = K(t-t_1)$$

which is derived from the equation expressing the speed of a mono-

molecular autocatalytic reaction, as in the case of the bacterial action alone. The values of K and t_1 calculated from these figures are shown in Table 62. Even when bacteria act along with autolytic enzymes these values are approximately the same as those in the case of bacterial action alone. However, the value of A/a varies in each other. In the case where bacteria were allowed to develop in the muscle containing autolytic enzymes A/a is larger than that in the bacterial action only.

Table 58. Chub mackerel.

Time of incubation	Bact. action only			Combi. act. of enz.-bact.			Enz. only	
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y
days	mg.	mg.		mg.	mg.		mg.	mg.
0	0.473			0.473			0.473	
3	—	—	—	0.473	0		0.526	0.053
5	0.701	0.228	1.055	0.596	0.123	2.523	0.561	0.088
7	1.016	0.543	1.506	1.103	0.630	1.297	0.596	0.123
9	1.471	0.998	1.905	2.031	1.553	1.840	0.613	0.140
11	1.926	1.453	0.267	3.238	2.765	0.421	0.613	0.140
12	2.188	1.715	0.514	3.763	3.290	0.797	—	—
14	2.538	2.065	1.073	4.113	3.640	1.318	0.613	0.140
16	2.713	2.240		4.288	3.815		—	—
19	2.713	2.240		4.288	3.815		0.630	0.157

Table 59. Sea bream.

Time of incubation	Bact. action only			Combi. act. of enz.-bact.			Enz. only	
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y
days	mg.	mg.		mg.	mg.		mg.	mg.
0	0.280			0.280			0.280	
2	—	—		0.315	0.035	3.892	0.368	0.088
4	0.315	0.035	3.997	—	—	—	0.385	0.105
6	0.420	0.140	2.612	0.490	0.210	2.687	0.385	0.105
8	0.578	0.298	2.961	0.875	0.595	1.180	0.403	0.123
9	0.700	0.420	1.126	1.453	1.173	1.544	—	—
11	1.225	0.945	1.557	2.415	2.135	1.950	0.420	0.140
13	2.275	1.995	0.103	3.675	3.395	0.475	—	—
14	2.888	2.608	0.434	4.200	3.920	0.806	—	—
16	3.675	3.395	1.288	4.725	4.445	1.704	—	—
18	3.850	3.570		4.813	4.533		0.420	0.140
20	3.850	3.570		4.813	4.533		—	—

Table 60. Spiny lobster.

Time of incubation	Bact. action only			Combi. act. enz-bact.			Enz. only	
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y
days	mg.	mg.		mg.	mg.		mg.	mg.
0	1.120			1.120			1.120	
2	1.225	0.105	2.800	1.278	0.158	2.774	1.243	0.123
3	1.400	0.280	1.273	1.645	0.525	1.360	1.278	0.158
5	1.750	0.630	1.744	2.450	1.330	1.953	—	—
6	1.925	0.805	1.923	2.800	1.680	0.170	—	—
7	2.100	0.980	0.095	2.985	1.865	0.293	—	—
8	2.363	1.243	0.375	3.238	2.118	0.481	1.305	0.185
9	2.538	1.418	0.608	3.500	2.380	0.735	—	—
10	2.888	1.768		3.763	2.643	1.180	—	—
12	2.888	1.768		3.850	2.730	1.492	—	—
14	2.888	1.768		3.938	2.818		—	—

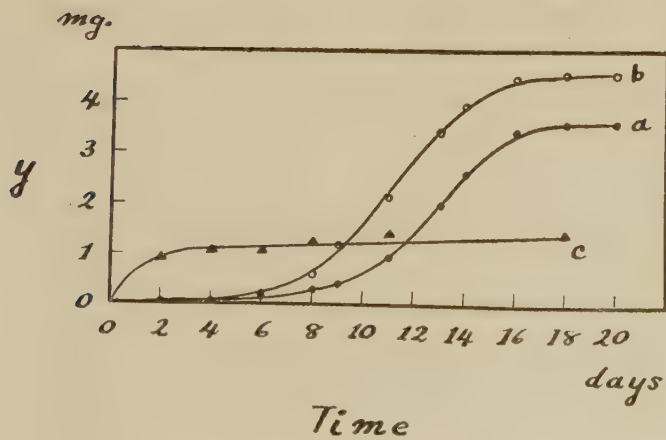
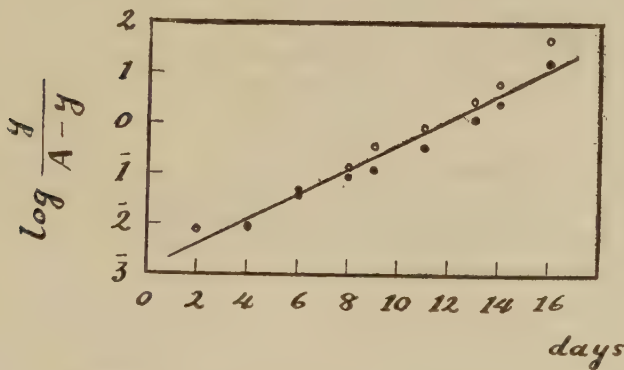


Fig. 32. Influence of autolytic enzymes on the bacterial decomposition. (in the case of sea bream). a, b and c are the same in Fig. 31.

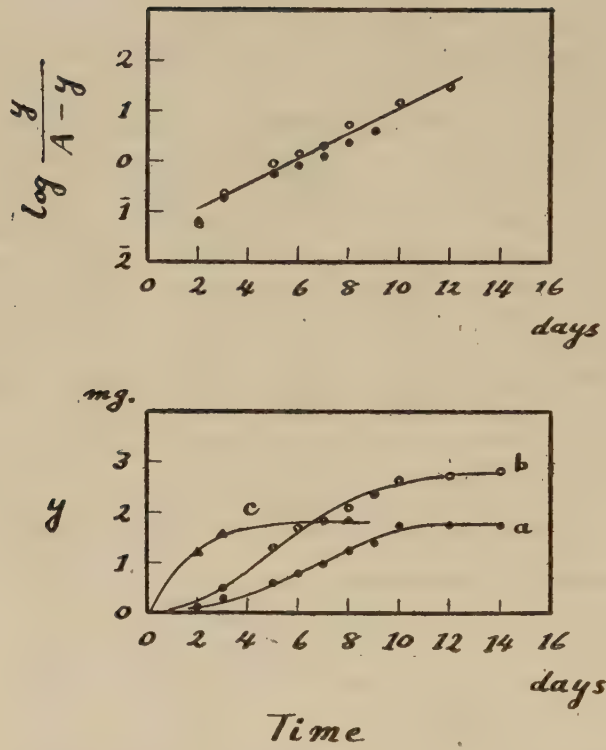


Fig. 33. Influence of autolytic enzymes on the bacterial decomposition (in the case of spiny lobster).

a, b and c are the same in Fig. 31.

Table 6L. Growth of bacteria.

	0 h.	23 hs.	48 hs.	72 hs.
(chub mackerel).				
Bact. only	} 721	*248 × 10 ⁴	+255 × 10 ⁶	218 × 10 ⁷
Bact. and enz.		*265 × 10 ⁴	+308 × 10 ⁶	163 × 10 ⁷
(sea bream)				
Bact. only	} 453	194 × 10 ⁵	362 × 10 ⁶	133 × 10 ⁷
Bact. and enz.		183 × 10 ⁵	243 × 10 ⁶	118 × 10 ⁷
(spiny lobster)				
Bact. only	} 662	228 × 10 ⁵	382 × 10 ⁶	113 × 10 ⁷
Bact. and enz.		218 × 10 ⁵	326 × 10 ⁶	216 × 10 ⁷

*.....20 hours, +.....44 hours.

Therefore, it is evident that the amount of the bacterial decomposition of muscles containing autolytic enzymes is always more than that of the other during the same length of time after the inoculation of bacteria.

Table 62. Values of various constants.

		a	A	A/a	K	t_1
		mg.	mg.			d.
Chub mackerel	Bact. only	17.942	2.240	0.125	0.245	9.60
	Bact. and enz.		3.815	0.212	0.275	9.50
Sea bream	Bact. only	14.306	3.570	0.249	0.250	11.60
	Bact. and enz.		4.533	0.318	0.250	11.60
Spiny lobster	Bact. only	7.686	1.768	0.236	0.250	5.60
	Bact. and enz.		2.818	0.366	0.250	5.60

The rate of growth of bacteria does not seem to be influenced by the autolytic enzymes contained in the medium as shown in Table 61.

It is a well known fact that the progress of enzymic decomposition obeys the law of monomolecular chemical reaction, and satisfies the equation:

$$\frac{dy}{dt} = K(a-y) \quad \text{or} \quad K = \frac{1}{t} \ln \frac{a}{a-y}$$

Therefore, in the action of enzymes only, the decomposition can not be compared with the bacterial decomposition which does not obey the equation. But it will be understood that when autolytic enzymes act only, the decomposition is very smaller than in the bacterial decomposition.

IV. Summary

In the case of bacterial decomposition of meat of aquatic animals, the increase of autolytic products produced in the meat has little influenced upon the values of K and A/a , if the amount of autolytic products is not very much, while, the value of t_1 has a tendency to become smaller with increasing amount of the autolytic products, that is, the inductive period becomes shorter. In other words, the time required for attaining the phase of rapid decomposition (i.e. length of the inductive period), becomes shorter with the length of time autolysis has gone on, but after this time (i.e. at the end of the inductive period) the speed of the decomposition is almost constant irrespective of the degree of autolysis.

In the case of combined action of both bacteria and autolytic enzymes, both the values of K and t_1 are approximately the same as those in the case of bacterial action only, although the value of A/a in the former case is larger than in the latter. Therefore, in the same length of time after inoculation of bacteria, the decomposition in the former is larger than that in the latter. From this fact it is presumed that raw meat is decomposed by bacteria more than heated meat.

The growth of bacteria is not almost influenced by both the amount of autolytic products and autolytic enzymes.

From the above mentioned facts, the effect of autolysis on the bacterial decomposition of meat of aquatic animals may be concluded as follows:

The time required for occurring the rapid bacterial decomposition, becomes shorter by the increase of autolytic products, and the degree of the decomposition becomes larger by the action of autolytic enzymes, whereas the speed of the decomposition is not influenced.

PART FOUR

Effects of Physical and Chemical Changes of the Environment.

It seems to be of great significance in preservation and manufacturing of aquatic products, to ascertain how the effect is exercised on the bacterial decomposition of meat of aquatic animals in consequence of physical and chemical changes of environment. However, we have now a little knowledge about this problem only. And this is the reason why the author takes it up in the present study.

I. Influence of Temperature.

Both the bacterial growth and the bacterial action are to be influenced by temperature. The effect of temperature upon the bacterial growth has been studied by many investigators. It is well known that at temperature lower than the optimum growth temperature, the growth of bacteria becomes slower with the lowering of temperature and that the effect is defined by the ARRHENIUS' equation. The temperature coefficient Q_{10} is not only different according to the kind of bacteria but also to the range of temperature and generally it becomes larger with the lowering temperature. Water bacteria, playing an important rôle on the spoilage of fishes, are tolerant to cold and the optimum growth temperature lies between 20°C and 25°C.⁽¹⁰⁾ In an investigation on the relation between the growth of *Pseudomonas fluorescens* and temperature (below 20°C), HESS⁽⁴⁹⁾ observed that the temperature coefficient, Q_{10} , is 3.7 between 20°C and 5°C, 8.4 between 5°C and 0°C, and 9.3 between 0°C and -3°C, and that the generation time is the shortest at 20°C.

On the relation between the spoilage of meat of aquatic animals and temperature, there is comparatively few investigation.

In the present study, the author has examined, using *Pseudomonas fluorescens*, *Serratia marcescens* and the meat extract of chub mackerel, the progress of the bacterial decomposition at various temperatures.

Experiment.

Twenty five cubic centimeters of the solution of the meat extract prepared by the above mentioned method were pipetted into a test

Table 63. continued.

Time of incubation	15°C			11.5°C			8.5°C		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.315			0.315			0.315		
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—
5	0.350	0.035	2.971	—	—	—	—	—	—
6	0.368	0.053	1.160	—	—	—	—	—	—
7	0.378	0.063	1.246	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—
9	0.438	0.123	1.618	—	—	—	—	—	—
10	0.473	0.158	1.781	—	—	—	—	—	—
11	0.508	0.193	1.930	0.350	0.035	2.971	—	—	—
12	0.560	0.245	0.146	—	—	—	—	—	—
13	0.613	0.298	0.389	—	—	—	—	—	—
14	0.648	0.333	0.583	—	—	—	—	—	—
15	0.665	0.350	0.700	0.368	0.053	1.160	0.315	0	—
16	0.683	0.368	0.850	—	—	—	—	—	—
18	—	—	—	0.438	0.123	1.618	0.333	0.018	2.652
20	—	—	—	0.473	0.158	1.781	0.350	0.035	2.971
22	—	—	—	0.525	0.210	0.000	0.368	0.053	1.160
25	—	—	—	0.595	0.280	0.301	0.385	0.070	1.301
27	—	—	—	—	—	—	0.403	0.088	1.424
29	—	—	—	—	—	—	0.438	0.123	1.618

Note: At 41.5°C, organisms were killed.

Table 64. Influence of temperature on the bacterial decomposition.
(by the action of *Serratia marcescens*).

[illegible]

Table 64. continued.

Time of incubation	24°C			20.5°C		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.315			0.315		
2	0.315	0		—	—	—
4	0.333	0.018	2.582	0.315	0	
5	0.350	0.035	2.887	—	—	—
6	0.360	0.045	1.005	—	—	—
7	0.368	0.053	1.084	0.350	0.035	2.887
8	0.420	0.105	1.436	0.378	0.063	1.169
9	0.438	0.123	1.526	0.420	0.105	1.436
10	0.490	0.175	1.746	0.438	0.123	1.526
12	0.630	0.315	0.256	0.490	0.175	1.746
14	0.735	0.420	0.779	0.613	0.298	0.191
16	0.770	0.455	1.114	0.700	0.385	0.565
18	—	—	—	0.735	0.420	0.779
20	0.805	0.490		—	—	—

Table 64. continued.

Time of incubation	17°C			14°C		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.315			0.315		
2	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—
7	—	—	—	—	—	—
8	—	—	—	—	—	—
9	0.385	0.070	1.222	0.333	0.018	2.582
10	0.403	0.088	1.341	0.350	0.035	2.887
12	0.455	0.140	1.603	0.368	0.053	1.084
14	0.508	0.193	1.813	0.385	0.070	1.222
16	0.578	0.263	0.065	0.420	0.105	1.436
18	0.648	0.333	0.327	—	—	—
20	0.700	0.385	0.565	0.490	0.175	1.746

Note: At 41°C and 9°C, organisms can be allowed to develop, but the decomposition does not take place.

tube (dia., 27 mm.; length, 215 mm.), sterilized and inoculated with bacteria and then incubated at various temperatures. At definite in-

Table 65. Growth of *Pseudomonas fluorescens*.

Temp. °C	0 h.	20 hs.	44 hs.	3 d.	4 d.	6 d.
34.5	162	167×10	—	387×10^5	281×10^5	313×15^5
31.5		883×10^5	249×10^6	512×10^6	—	—
27.0		768×10^5	306×10^6	998×10^6	—	—
23.5		295×10^5	102×10^7	459×10^7	—	418×10^7
21.5		106×10^5	289×10^6	104×10^7	—	578×10^7
18.5		501×10^3	164×10^6	548×10^6	103×10^7	187×10^7
15.0		572×10^2	118×10^6	576×10^6	107×10^7	197×10^7
11.5		160×10	617×10^4	153×10^6	382×10^6	728×10^6
8.5		623	712×10^2	139×10^5	579×10^5	192×10^6

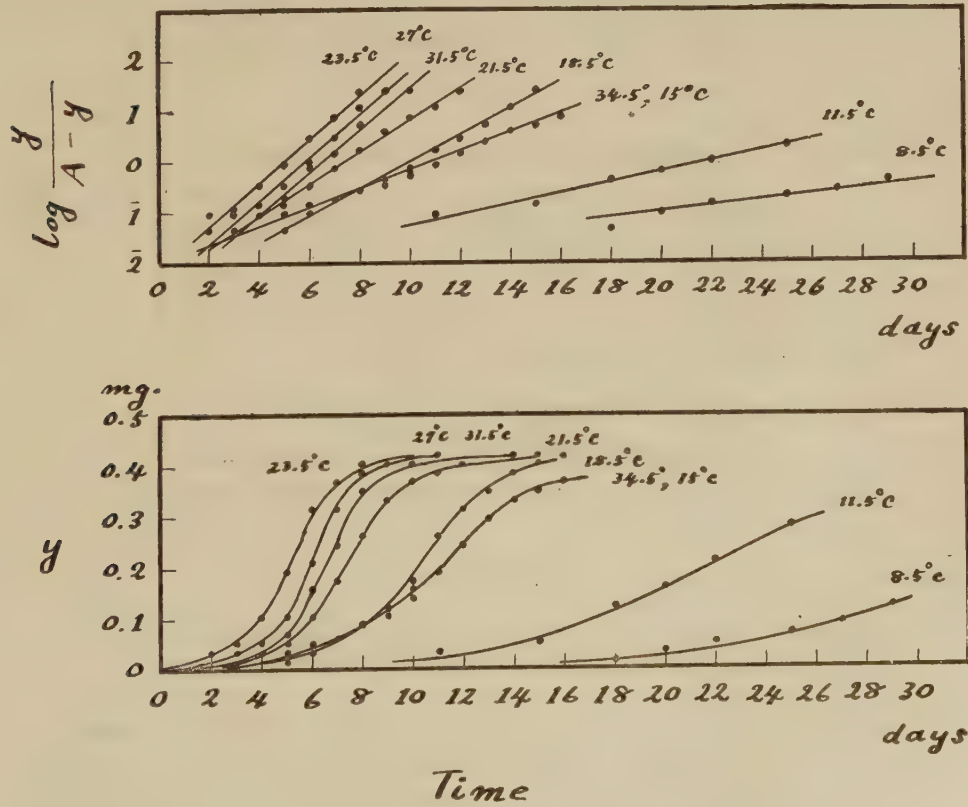


Fig. 34. Influence of temperature upon the bacterial decomposition.
(by the action of *Pseudomonas fluorescens*).

tervals, amino and ammonium nitrogen and bacterial counts in 1 c.c. were determined.

Results and Discussion.

Results obtained are shown in Tables 63-66. Figs. 34 and 35 are drawn from these data. The values of K and t_1 calculated from these figures are given in Tables 67 and 68.

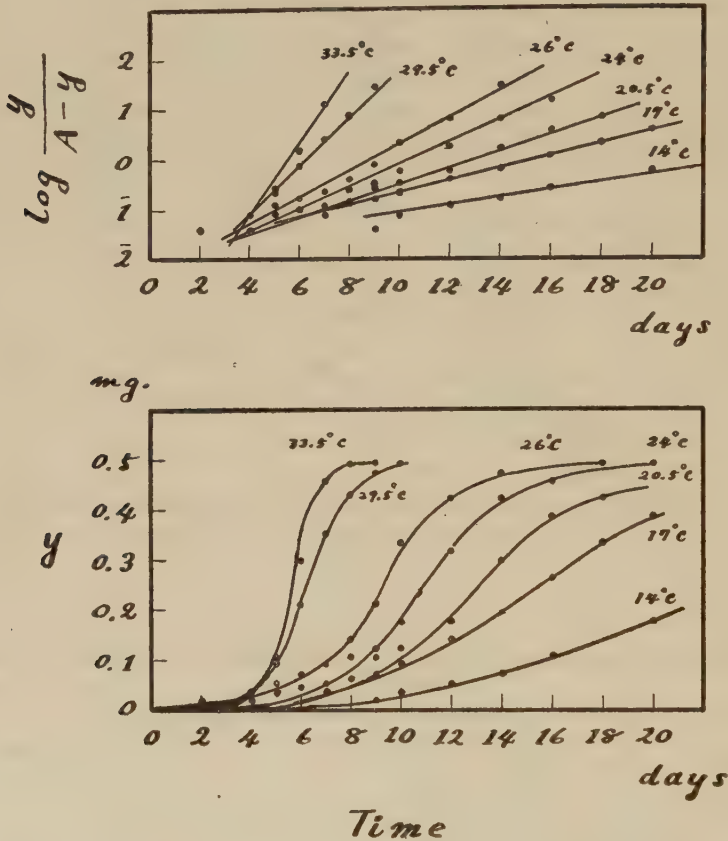


Fig. 35. Influence of temperature upon the bacterial decomposition.
(by the action of *Serratia marcescens*).

Both K and t_1 depend on the temperature, K varying conversely with t_1 . When the value of K is the greatest, the value of t_1 is the smallest, where the bacterial decomposition seems to have the optimum temperature. Below the optimum temperature, the length of the inductive period becomes longer and the speed of the decom-

Table 66. Growth of *Serratia marcescens*.

Temp. °C	0 h.	24 hs.	48 hs.	72 hs.
41.0	465	147×10^6	628×10^6	328×10^6
36.5		137×10^7	382×10^7	268×10^7
33.5		112×10^7	204×10^7	258×10^7
29.5		732×10^6	189×10^7	256×10^7
26.0		882×10^6	151×10^7	202×10^7
24.0		362×10^6	105×10^7	147×10^7
20.5		534×10^6	728×10^6	117×10^7
17.0		638×10^3	328×10^6	103×10^7
14.0		213×10^2	182×10^6	162×10^6
9.0		—	101×10^2	225×10^2

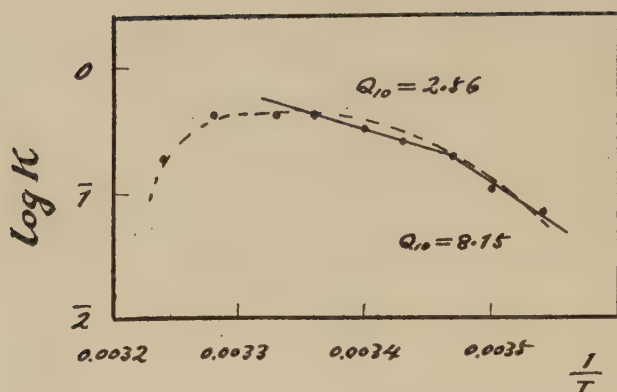
Table 67. Values of various constants.
(by *Pseudomonas fluorescens*)

Temp. °C	α	A	A/α	K	t_1
	mg.	mg.			d.
34.5	1.935	0.420	0.217	0.185	10.90
31.5				0.415	6.55
27.0				0.420	5.80
23.5				0.420	5.00
21.5				0.315	7.40
18.5				0.260	10.00
15.0				0.185	10.90
11.5				0.105	22.00
8.5				0.070	36.00

Table 68. Values of various constants.
(by *Serratia marcescens*)

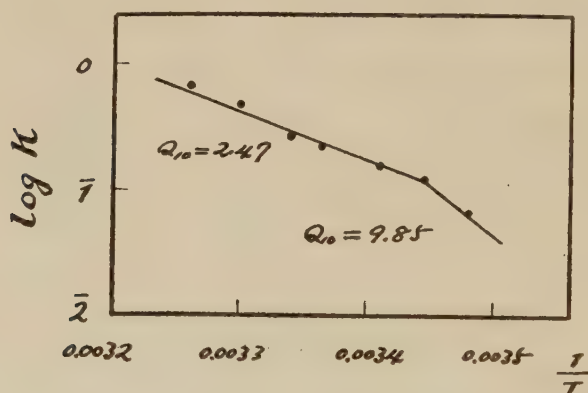
Temp. °C	α	A	A/α	K	t_1
	mg.	mg.			d.
33.5	1.838	0.490	0.261	0.715	5.50
29.5				0.485	6.35
26.0				0.265	8.95
24.0				0.220	10.40
20.5				0.160	13.10
17.0				0.120	15.50
14.0				0.070	24.20

position, defined by K , becomes slower with decreasing temperature. The relation between the logarithms of K and the reciprocals of absolute temperatures is shown in Figs. 36 and 37. It seems to be



Reciprocal of absolute temperature

Fig. 36. Influence of temperature upon the bacterial decomposition. Relation of the logarithms of the constant K to the reciprocals of the absolute temperatures. (by the action of *Pseudomonas fluorescens*).



Reciprocal of absolute temperature

Fig. 37. Influence of temperature upon the bacterial decomposition. Relation of the logarithms of the constant K to the reciprocals of the absolute temperatures. (by the action of *Serratia marcescens*).

linear within a certain definite range of the temperatures below the optimum, that is, the temperature coefficient Q_{10} is the same within this range. The value of Q_{10} calculated from the figures is 2.56 for the action of *Pseudomonas fluorescens* and 2.47 for *Serratia marcescens*. However, the inclination increases suddenly at a certain definite temperature which is about 15°C for the former and at about 17°C

for the latter. Therefore, Q_{10} is 8.15 for the former and 9.85 for the latter below that temperature.

The value of A/a is independent of temperature.

From the above mentioned fact, it is evident that the bacterial decomposition (by the action of *Pseudomonas fluorescens*) advances the most rapidly at about 25°C, and the degree of the bacterial decomposition within the same length of time decreases with lowering of temperature and that the degree of the decrease is rather remarkable at temperatures lower than 15°C.

Injurious effect is given on the bacterial growth by the lowering of temperature, although the degree of the effect is not remarkable than that on the bacterial decomposition, namely the degree of the effect upon the bacterial growth is not equal to that upon the bacterial action and the latter is more remarkable than the former, as shown in Tables 65 and 66.

From these facts it is presumed that at low temperature bacteria can develop, although can not decompose the meats actively.

II. Influence of the Surface Tension.

The relation of surface tension to bacterial development has hitherto been reported by several investigators. ^(2, 6, 21, 22, 30, 44, 67, 109, 117) But, so far, the relation between the bacterial decomposition of the medium and its surface tension is not known well.

The present chapter embodies the observations on the effect of surface tension upon the bacterial decomposition of the solution of meat extract of chub mackerel.

Experiment.

Fifty cubic centimeters of the solution of the meat extract prepared by the above mentioned method were put into several flasks of the capacity of 200 c.c. Various amounts (ranging from 0.01 to 0.0005%) of Na-recinoleate as the depressant were added to each flask, which was filled up to 100 c.c. with distilled water and then sterilized. These flasks were inoculated with *Pseudomonas fluorescens* or *Serratia marcescens* and incubated at 26°C. At regular intervals, amino and ammonium nitrogen in 5 c.c. and the surface tension of the medium were measured.

There are various materials to be used as the depressant. However, the material, showing the effect in very small quantity and not giving

the influence on bacteria in the above mentioned range, is the best suitable depressant. Such material is Na-recinoleate.⁽¹⁰⁹⁾

Measuring of the surface tension :—

The surface tension was measured using a torsion balance and was calculated from the following equation :

$$T_g = \frac{W}{2\pi(r_1 + r_2)} \qquad T_d = T_g \times 980$$

- T_g = surface tension (unit.....g./cm.).
 W = the value (gram) obtained by the torsion balance.
 r_1, r_2 = exterior and interior diameter of platinum ring used
in the torsion balance = 1.31 cm. and 1.24 cm.
 T_d = surface tension (unit.....dyne/cm.).

Results and Discussion.

Results obtained are listed in Tables 69–71. Figs. 38 and 39 are obtained from the data. Both K and t_1 calculated from these figures are shown in Table 72.

The surface tension changes with the decomposition of the

Table 69. Change of the surface tension
(by *Pseudomonas fluorescens*)

Time of incubation	Na-recinoleate %					
	0.01	0.005	0.0025	0.001	0.0005	0
days	dyne/cm.	dyne/cm.	dyne/cm.	dyne/cm.	dyne/cm.	dyne/cm.
0	37.43	39.03	40.25	41.72	46.59	50.04
2	39.89	40.86	42.94	44.78	49.67	53.10
3	44.17	46.37	50.04	56.40	61.91	61.91
4	47.59	50.16	52.49	55.06	57.63	57.63
5	49.06	50.28	51.51	50.90	50.90	50.90
7	50.28	50.28	50.65	50.90	49.06	49.06
8	51.51	51.51	50.90	50.90	50.90	50.90

(by *Serratia marcescens*)

0	37.43	39.03	40.25	41.72	—	50.04
1	40.86	42.70	44.17	45.76	—	50.04
2	44.17	45.39	46.37	47.23	—	48.20
3	44.17	45.39	45.76	45.39	—	45.39
4	42.91	—	44.17	44.79	—	43.92
5	42.91	—	43.56	42.91	—	42.91
7	42.91	—	—	—	—	—

Table 70. Influence of the initial surface tension on the bacterial decomposition (by *Pseudomonas fluorescens*).

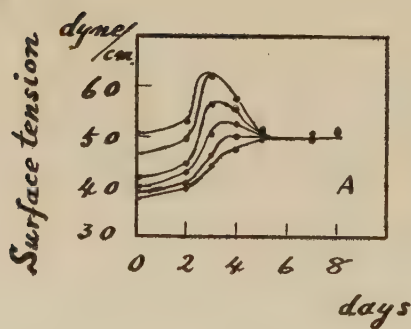
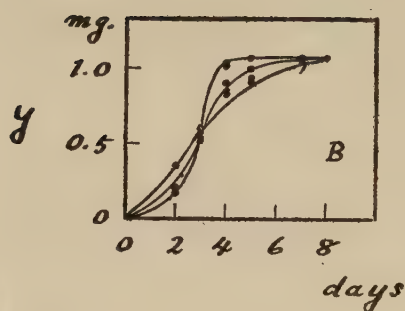
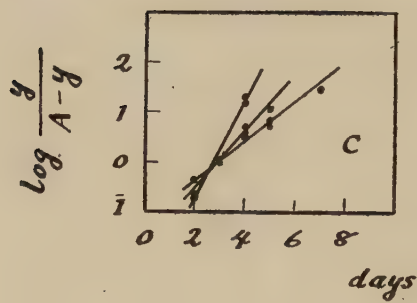
Time of incubation	37.43 dyne/cm.			39.03 dyne/cm.			40.25 dyne/cm.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.945			0.945			0.945		
2	1.120	0.175	1.285	1.120	0.175	1.285	1.155	0.210	1.381
3	1.523	0.578	0.057	1.470	0.525	1.972	1.540	0.595	0.084
4	1.978	1.033	1.299	1.960	1.015	1.162	1.855	0.910	0.716
5	2.030	1.085		2.030	1.085		1.943	0.998	1.060
7	2.030	1.085		2.030	1.085		2.030	1.085	
8	2.030	1.085		2.030	1.085		2.030	1.085	

Table 70. continued.

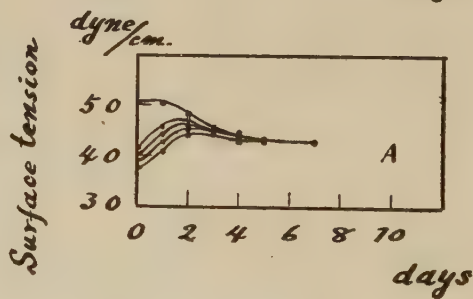
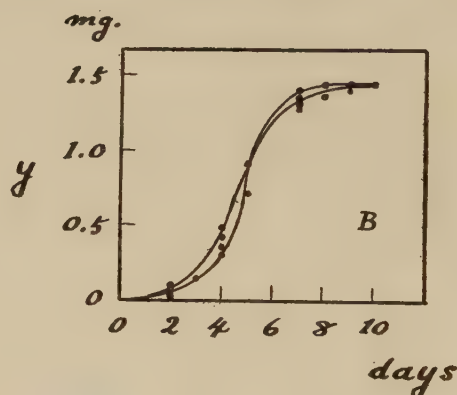
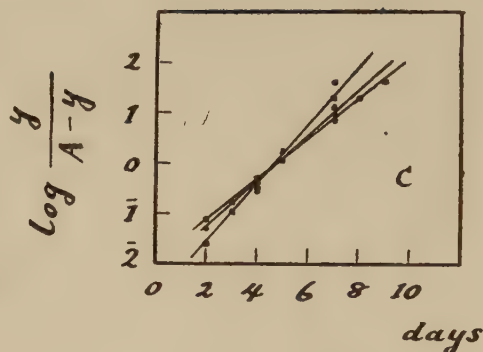
Time of incubation	41.72 dyne/cm.			46.59 dyne/cm.			50.04 dyne/cm.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.945			0.945			0.945		
2	1.155	0.210	1.381	1.295	0.350	1.678	1.295	0.350	1.678
3	1.470	0.525	1.972	1.540	0.595	0.084	1.540	0.595	0.084
4	1.768	0.823	0.497	1.785	0.840	0.536	1.803	0.858	0.586
5	1.890	0.945	0.830	1.855	0.910	0.717	1.873	0.928	0.772
7	2.030	1.085		1.995	1.050	1.478	1.995	1.050	1.478
8	2.030	1.085		2.030	1.085		2.030	1.085	

Table 71. Influence of the initial surface tension on the bacterial decomposition (by *Serratia marcescens*).

Time of incubation	37.43 dyne/cm.			39.03 dyne/cm.			40.25 dyne/cm.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.945			0.945			0.945		
1	0.945	0		0.945	0		0.945	0	
2	0.980	0.035	2.398	1.015	0.070	2.711	0.980	0.035	2.398
3	1.085	0.140	1.034	1.085	0.140	1.034	1.085	0.140	1.034
4	1.435	0.490	1.716	1.365	0.420	1.618	1.295	0.350	1.510
5	1.855	0.910	0.239	1.855	0.910	0.239	1.715	0.770	0.064
7	2.310	1.365	1.291	2.345	1.400	1.603	2.275	1.330	1.103
8	2.380	1.435		2.380	1.435		2.310	1.365	1.291
9	2.380	1.435		2.380	1.435		2.380	1.435	



Time



Time

Fig. 38. Influence of the surface tension on the bacterial decomposition (by the action of *Pseudomonas fluorescens*).

A.....change of the surface tension.

B.....production of amino and ammonium N.

C.....relation between $\log \frac{y}{A-y}$ and time.

Fig. 39. Influence of the surface tension on the bacterial decomposition (by the action of *Serratia marcescens*). A, B and C are the same in Fig. 38.

Table 71. continued.

Time of incubation	41.72 dyne/cm.			50.04 dyne/cm.		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.945			0.945		
1	0.945	0		0.945	0	
2	1.015	0.070	2.711	1.050	0.105	2.898
3	1.085	0.140	1.034	1.155	0.210	1.235
4	1.260	0.315	1.450	1.330	0.385	1.565
5	1.715	0.770	0.064	1.715	0.770	0.064
7	2.240	1.295	0.963	2.205	1.260	0.858
8	2.310	1.365	1.291	2.310	1.365	1.291
9	2.380	1.435		2.345	1.400	1.603
10	—	—		2.380	1.435	

Table 72. Values of various constants (*Pseudomonas fluorescens*).

Surf. tension	a	A	A/a	K	t ₁
dyne/cm.	mg.	mg.			d.
37.43	4.830	1.035	0.225	0.975	2.80
39.03				0.975	2.80
40.25				0.570	2.90
41.72				0.395	2.90
46.59				0.395	2.90
50.04				0.395	2.90

(*Serratia marcescens*).

37.43	4.830	1.435	0.276	0.575	4.70
39.03				0.575	4.70
40.25				0.450	4.80
41.72				0.400	4.80
50.04				0.400	4.80

medium, but ceases to change when the process of the decomposition reaches equilibrium, and at this time it becomes almost constant irrespective of the different initial surface tension.

The value of *K* depends on the initial surface tension of the medium and increases with depression of the initial surface tension. But both *A/a* and *t*₁ are almost independent of the surface tension.

From this fact it is concluded that in the case of the depression of the initial surface tension, the degree of the bacterial decomposition

is smaller in the early stage, but is rapidly increases along the progress of the decomposition, and the decomposition is completed rapidly (i.e. is reached to the equilibrium).

It is not clear whether those effects are due to the surface tension or to the chemical nature of the depressant. But these effects seem to be due to the former rather than to the latter, within the limits of concentration of the depressant employed.

III. Influence of Halogen Ions.

On the effect on physiological activities of bacteria of halogen ions excepting Cl^- , few papers seem to have been published. Various authors have reported that Cl^- has very weak toxic action and in general its effect is negligible. Inhibitory action of NaCl in higher concentration is known to be due to the osmotic pressure. F^- is the most active among the halogen ions. According to EFFRONT,⁽²⁴⁾ F^- in hydrofluoric acid must be potent, as this acid is 10–20 times as inhibitory as hydrochloric acid. MEYERHOF⁽⁷²⁾ has studied the effect of halogen ions on the respiration of nitrifying bacteria and found that 0.05 N NaF reduced the respiration by 30% and 0.1 N by 65%, 0.1 N NaBr reduced by 27% and 0.2 N by 56%, 0.05 N NaI reduced by 44% and 0.1 N by 57%. ISCHIKAWA⁽⁶⁰⁾ found that iodides even in low concentrations tend to inhibit ammonia formation by proteolytic as well as urea bacteria; no stimulation was evidenced. According to BOAS⁽¹²⁾ NaI or KI in higher concentration than about 0.3 M inhibit the growth of bacteria, but not the growth of molds. He found also that NaF in concentration of 0.033–0.0033 M stimulate the growth of yeast, but with the same compound of a concentration 0.000008 M the growth is worse than the control.

In the present experiment the effect of halogen ions on the bacterial decomposition of fish muscle (chub mackerel) was studied, with NaF , NaI , NaBr and NaCl which have the same cation.

Experiment.

Fifty cubic centimeters of the muscle suspension prepared by the already mentioned method were put into a sterilized flask of the capacity of 200 c.c., and mixed each with certain M/2 halogenide solution (excepting 2 M NaCl used), the bacterial suspension of *Pseudomonas fluorescens* and sterilized distilled water, as shown in Table 73. And then these flasks were incubated at 26°C–28°C. After

Table 73. Composition of the decomposing mixtures.

No. of flask	I	II	III	IV	V	VI	VII	VIII	IX	X
Muscle suspension	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50	c.c. 50
Solution of halogenide	49	25	10	5	2	1	0.5	0.25	0.1	0
Distilled water	0	24	39	44	47	48	48.5	48.75	48.9	49
Bacterial suspension	1	1	1	1	1	1	1	1	1	1
Mol. concentration	0.245	0.125	0.05	0.025	0.01	0.005	0.0025	0.00125	0.0005	0

Note: NaCl used was 2 M, and therefore its concentration is 4 times as great.

standing for a definite time, volatile basic nitrogen in 10 c.c. and bacterial counts in 1 c.c. were determined.

Results.

Results obtained are given in Tables 74–77. The generation time, g and the velocity coefficient of the rate of the bacterial growth, k were calculated, during the first 24 hours, by the equation previously described (Part Two, Chapter II). The growth curves plotted from Table 74 are shown in Fig. 40.

Influence of NaF:—At a lower concentration than 0.00125 M, the effect on the growth rate of bacteria is negligible. But, the growth rate is decreased at higher concentration than 0.0025 M and is inhibited at 0.05 M. The production of volatile basic nitrogen is decreased with increasing concentration above 0.0005 M. Volatile base is not formed at higher concentration than 0.05 M, because bacteria are not allowed to develop at this concentration. Both the bacterial growth and the bacterial decomposition are not stimulated by this salt within the range of concentrations employed.

Influence of NaI:—The growth rate is slightly stimulated in 0.0025 M–0.01 M solution, but not influenced at lower concentration than that. The growth is inhibited at higher concentration than 0.245 M. Production of volatile base is the greatest at the concentration of 0.00125 M, and decreases at higher concentration than 0.005 M.

Influence of NaBr:—Effect on the rate of the growth is almost negligible at lower concentration than 0.05 M. The growth rate decreases at higher concentration than 0.05 M. The formation of volatile base increases at 0.00125 M, but becomes less at higher concentration than 0.025 M.

Influence of NaCl:—Concentration of 0.1 M–0.01 M slightly

Table 74. Bacterial growth in the medium containing various concentrations of halogenides.

Time of incubation	I	II	III	IV	V	VI	VII	VIII	IX	X
(NaF)										
days	615	615	615	615	615	615	615	615	615	615
0	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—
(NaI)										
0	611	611	611	611	611	611	611	611	611	611
1	235	188 × 10 ³	708 × 10 ⁵	197 × 10 ⁶	395 × 10 ⁶	379 × 10 ⁶	385 × 10 ⁶	201 × 10 ⁶	218 × 10 ⁶	163 × 10 ⁶
2	0	302 × 10 ⁵	375 × 10 ⁶	813 × 10 ⁶	963 × 10 ⁶	935 × 10 ⁶	965 × 10 ⁶	924 × 10 ⁶	—	—
3	0	107 × 10 ⁶	104 × 10 ⁷	137 × 10 ⁷	157 × 10 ⁷	179 × 10 ⁷	156 × 10 ⁷	138 × 10 ⁷	131 × 10 ⁷	114 × 10 ⁷
(NaBr)										
0	636	636	636	636	636	636	636	636	636	636
1	821 × 10 ⁴	860 × 10 ⁵	215 × 10 ⁶	203 × 10 ⁶	205 × 10 ⁶	193 × 10 ⁶	226 × 10 ⁶	251 × 10 ⁶	173 × 10 ⁶	182 × 10 ⁶
2	382 × 10 ⁶	560 × 10 ⁶	980 × 10 ⁶	979 × 10 ⁶	982 × 10 ⁶	995 × 10 ⁶	983 × 10 ⁶	996 × 10 ⁶	108 × 10 ⁷	118 × 10 ⁷
(NaCl)										
0	635	635	635	635	635	635	635	—	—	635
1	—	708 × 10 ⁴	129 × 10 ⁶	366 × 10 ⁶	358 × 10 ⁶	356 × 10 ⁶	368 × 10 ⁶	—	—	203 × 10 ⁶
2	—	615 × 10 ⁶	125 × 10 ⁷	154 × 10 ⁷	141 × 10 ⁷	201 × 10 ⁷	109 × 10 ⁷	—	—	115 × 10 ⁷
3	980 × 10	823 × 10 ⁶	117 × 10 ⁷	153 × 10 ⁷	172 × 10 ⁷	222 × 10 ⁷	137 × 10 ⁷	—	—	138 × 10 ⁷

Table 75. The generation time and the velocity coefficient of the rate of growth of bacteria.

No. of flask	NaF		NaI		NaBr		NaCl	
	<i>g</i>	<i>k</i>	<i>g</i>	<i>k</i>	<i>g</i>	<i>k</i>	<i>g</i>	<i>k</i>
I	hrs.	—	hrs.	—	hrs.	0.39	hrs.	—
II	—	—	2.90	0.23	1.41	0.49	1.79	0.39
III	—	—	1.43	0.49	1.31	0.53	1.36	0.51
IV	16.65	0.04	1.31	0.53	1.31	0.53	1.25	0.56
V	4.11	0.17	1.24	0.56	1.31	0.53	1.26	0.55
VI	2.48	0.28	1.25	0.56	1.32	0.53	1.26	0.55
VII	1.61	0.43	1.25	0.56	1.30	0.53	1.25	0.56
VIII	1.34	0.52	1.31	0.53	1.29	0.54	—	—
IX	1.34	0.52	1.30	0.53	1.33	0.52	—	—
X	1.32	0.53	1.33	0.52	1.33	0.52	1.31	0.53

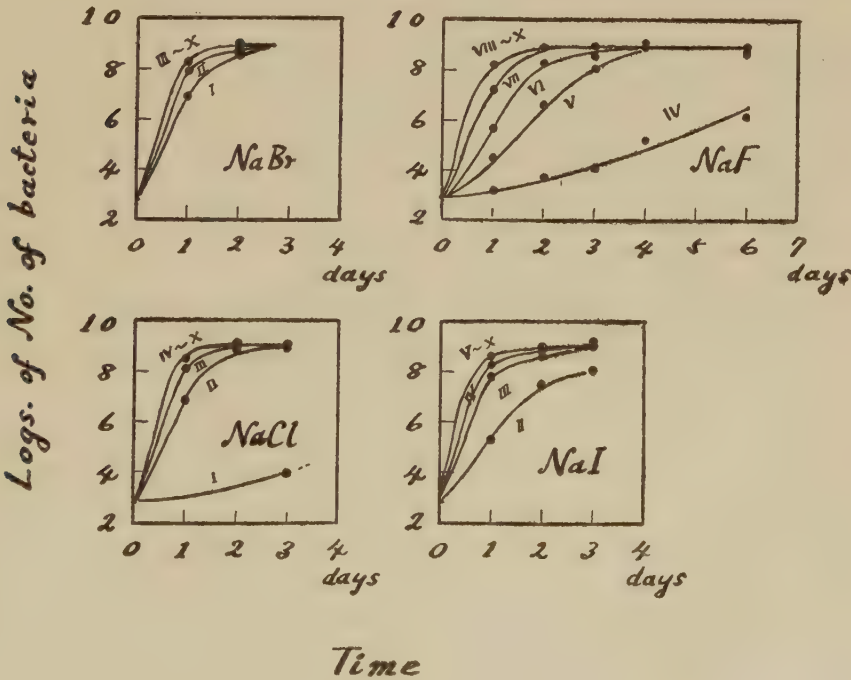


Fig. 40. Influence of Na-halogenides on the growth of bacteria in the muscle suspension

stimulates the growth rate and its activity remains constant within this limit. The growth is retarded at higher concentration than

Table 76. Volatile basic nitrogen produced in 5 days.

No. of flask	NaF		NaI		NaBr		NaCl	
	Determ.	Increas.	Determ.	Increas.	Determ.	Increas.	Determ.	Increas.
I	mg. —	mg. —	mg. 0.245	mg. 0	mg. 1.148	mg. 0.903	mg. 0.350	mg. 0.105
II	—	—	1.260	1.015	1.173	0.928	1.015	0.770
III	0.245	0	1.313	1.068	1.470	1.225	1.470	1.225
IV	0.245	0	1.435	1.190	1.505	1.260	1.523	1.278
V	0.805	0.560	1.558	1.313	1.610	1.365	1.628	1.383
VI	1.085	0.840	1.558	1.313	1.645	1.400	1.750	1.505
VII	1.400	1.155	1.610	1.365	1.733	1.488	1.838	1.593
VIII	1.540	1.295	1.680	1.435	1.785	1.540	—	—
IX	1.575	1.330	1.540	1.295	1.628	1.383	—	—
X	1.638	1.393	1.505	1.260	1.540	1.295	1.785	1.540

(at the beginning.....0.245 mg.)

Table 77. Volatile basic nitrogen produced in 20 days.

No. of flask	NaF		NaI		NaBr		NaCl	
	Determ.	Increas.	Determ.	Increas.	Determ.	Increas.	Determ.	Increas.
I	mg. —	mg. —	mg. 0.350	mg. 0.105	mg. 1.785	mg. 1.540	mg. 1.932	mg. 1.687
II	—	—	1.820	1.575	2.048	1.803	2.275	2.030
III	0.245	0	2.058	1.813	3.028	2.783	2.555	2.310
IV	1.593	1.348	3.360	3.115	3.343	3.098	3.500	3.255
V	2.065	1.820	4.130	3.885	3.798	3.553	3.623	3.378
VI	2.555	2.310	4.270	4.025	3.990	3.745	3.588	3.343
VII	2.835	2.590	4.270	4.025	4.130	3.885	3.535	3.290
VIII	2.905	2.660	4.568	4.323	4.183	3.938	—	—
IX	2.940	2.695	4.288	4.043	4.095	3.850	—	—
X	3.098	2.853	4.200	3.955	4.095	3.850	3.518	3.273

(at the beginning.....0.245 mg.)

that. Volatile base formation seems to be slightly increased near the concentration of 0.01 M.

From the results obtained it is understood that the inhibitory action of halogen ions is in the order $F' > I' > Br' > Cl'$.

IV. Influence of Alkali Metals and Alkali-Earth Metals.

Of the effects of alkali metals and alkali-earth metals upon the physiology of bacteria, the effects of Na, K, Ca and Mg have been

studied by various investigators, but the effects of Li, Rb, Cs, Sr,⁽⁵²⁾ Ba^(27, 52) and Be are rather poorly known. Most investigators have attacked certain peculiarities in relation to certain metals. Of the relation between these metals and the decomposition of fish muscle by bacteria our knowledge is still meagre.

In the present experiment, the effects of alkali metals and alkali-earth metals on the bacterial decomposition of fish muscle have been studied.

Experiment.

The same method as in Chapter III was used. Metal-salts used are chlorides, in which Cl⁻ has comparatively weak activity. Chlorides of alkali metal in concentration of 2 M and alkali-earth metal in concentration of 1 M were made, and sterilized. They were mixed with each other, as shown in Table 78. The initial pH-values of these mixtures were 6.2.

Table 78. Composition of the decomposing mixtures.

No. of flask		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
		c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Muscle suspension		50	50	50	50	50	50	50	50	50	50	50
Solution of salt		49	25	10	5	2	1	0.5	0.25	0.1	0.05	0
Distilled water		0	24	39	44	47	48	48.5	48.75	48.9	48.95	49
Bacterial suspension		1	1	1	1	1	1	1	1	1	1	1
Mol.	alkali m.	0.98	0.5	0.2	0.1	0.04	0.02	0.01	0.005	0.002	0.001	0
conc.	alkali-ear. m.	0.49	0.25	0.1	0.05	0.02	0.01	0.005	0.0025	0.001	—	0

Results.

Results obtained are given in Tables 79–85.

Influence of LiCl:—The rate of growth is slightly stimulated in concentration of 0.04–0.005 M, but not influenced at lower concentration than that and also decreased at higher concentration than 0.2 M. The growth is inhibited at higher concentration than 1.0 M. The decomposition seems to be very slightly stimulated at about 0.02–0.002 M, but not influenced at lower concentration than that, and decreases at higher concentration than that.

Influence of NaCl:—The growth rate is slightly stimulated at 0.1–0.01 M, and it remains constant within this range. The growth is retarded at higher concentration than that. The decomposition

Table 79. Influence of LiCl.

No. of flask	Bacterial growth (inoculum, 742)			Volatile basic nitrogen (at the beginning.....0.368 mg.)			
	No. of cells, after 25.5 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	298	hrs.	—	mg. 0.368	mg. 0	mg. 0.592	mg. 0.224
II	342 × 10 ³	2.91	0.24	1.453	1.085	2.136	1.768
III	293 × 10 ⁶	1.38	0.50	1.803	1.435	2.398	2.030
IV	636 × 10 ⁶	1.30	0.53	2.066	1.698	2.098	2.730
V	898 × 10 ⁶	1.27	0.55	2.206	1.838	—	—
VI	867 × 10 ⁶	1.27	0.55	2.363	1.995	4.026	3.658
VII	724 × 10 ⁶	1.29	0.54	2.346	1.978	4.218	3.850
VIII	112 × 10 ⁷	1.25	0.55	2.346	1.978	4.901	4.533
IX	513 × 10 ⁶	1.32	0.53	2.346	1.978	5.093	4.725
X	523 × 10 ⁶	1.31	0.53	2.311	1.943	4.603	4.235
XI	493 × 10 ⁶	1.31	0.53	2.258	1.890	4.726	4.358

Table 80. Influence of NaCl.

No. of flask	Bacterial growth (inoculum, 635)			Volatile basic nitrogen (at the beginning.....0.298 mg.)			
	No. of cells, after 24 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	886	hrs.	—	mg. 0.351	mg. 0.053	mg. 1.921	mg. 1.623
II	688 × 10 ⁴	1.80	0.39	1.016	0.718	2.276	1.978
III	133 × 10 ⁶	1.36	0.51	1.471	1.173	2.556	2.258
IV	356 × 10 ⁶	1.26	0.55	1.523	1.225	3.501	3.203
V	353 × 10 ⁶	1.26	0.55	1.628	1.330	3.623	3.325
VI	350 × 10 ⁶	1.26	0.55	1.751	1.453	3.588	3.290
VII	375 × 10 ⁶	1.25	0.56	1.838	1.540	3.536	3.238
XI	183 × 10 ⁶	1.32	0.53	1.786	1.488	3.518	3.220

seems to be slightly stimulated at about 0.01 M, and decreased in higher concentration than that.

Influence of KCl:—Concentration of 0.002–0.2 M stimulates the growth, that of about 0.1 M causing it at most. At higher concentration than 1.0 M the growth is inhibited. The decomposition seems to be slightly stimulated at 0.02–0.002 M, although it is not very clear. And also it decreases at higher concentration than that.

Table 81. Influence of KCl.

No. of flask	Bacterial growth (inoculum, 602)			Volatile basic nitrogen (at the beginning.....0.315 mg.)			
	No. of cells, after 22 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	0	hrs. —	—	mg. 0.315	mg. 0	mg. 0.420	mg. 0.105
II	613 × 10	6.58	0.11	1.173	0.858	2.118	1.803
III	235 × 10 ⁶	1.18	0.59	—	—	2.923	2.608
IV	795 × 10 ⁶	1.08	0.64	1.558	1.243	3.728	3.413
V	278 × 10 ⁶	1.17	0.59	—	—	3.938	3.623
VI	213 × 10 ⁶	1.19	0.58	1.645	1.330	4.323	4.008
VII	196 × 10 ⁶	1.20	0.58	—	—	4.620	4.305
VIII	135 × 10 ⁶	1.24	0.56	1.645	1.330	4.708	4.393
IX	863 × 10 ⁵	1.28	0.54	1.680	1.365	4.778	4.463
X	680 × 10 ⁵	1.31	0.53	—	—	4.620	4.305
XI	560 × 10 ⁵	1.33	0.52	1.575	1.260	4.813	4.498

Table 82. Influence of MgCl₂.

No. of flask	Bacterial growth (inoculum, 794)			Volatile basic nitrogen (at the beginning.....0.385 mg.)			
	No. of cells, after 23 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	0	hrs. —	—	mg. 0.385	mg. 0	mg. 0.385	mg. 0
II	289	—	—	0.385	0	0.385	0
III	182 × 10 ⁵	1.59	0.44	1.348	0.963	2.030	1.645
IV	421 × 10 ⁶	1.21	0.57	1.585	1.200	3.290	2.905
V	342 × 10 ⁶	1.23	0.56	1.925	1.540	5.898	5.513
VI	223 × 10 ⁶	1.27	0.55	1.785	1.400	6.370	5.985
VII	136 × 10 ⁶	1.32	0.53	1.803	1.418	6.108	5.723
VIII	124 × 10 ⁶	1.33	0.52	1.780	1.395	5.753	5.368
IX	112 × 10 ⁶	1.34	0.52	1.780	1.395	5.550	5.165
XI	125 × 10 ⁶	1.33	0.52	1.785	1.400	5.320	4.935

Influence of MgCl₂:—The growth is slightly stimulated at 0.05–0.01 M, decreased at higher concentration than that, and inhibited at higher concentration than 0.25 M. The decomposition increases at 0.02–0.005 M, but decreases at higher concentration than that.

Table 83. Influence of CaCl_2 .

No. of flask	Bacterial growth (inoculum, 825)			Volatile basic nitrogen (at the beginning.....0.280 mg.)			
	No. of cells, after 23 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	0	hrs.	—	mg.	mg.	mg.	mg.
II	0	—	—	0.280	0	0.280	0
III	460	—	—	0.280	0	0.683	0.403
IV	648×10^3	2.39	0.29	0.280	0	0.718	0.438
V	237×10^6	1.27	0.55	0.320	0.040	4.410	4.130
VI	319×10^6	1.24	0.56	1.678	1.398	9.485	9.205
VII	228×10^6	1.27	0.55	2.258	1.978	9.380	9.100
VIII	169×10^6	1.30	0.53	2.230	1.950	—	—
IX	171×10^6	1.30	0.53	2.450	2.170	6.825	6.545
XI	134×10^6	1.30	0.53	2.520	2.240	6.720	6.440
		1.33	0.52	2.345	2.065	4.865	4.585

Table 84. Influence of SrCl_2 .

No. of flask	Bacterial growth (inoculum, 823)			Volatile basic nitrogen (at the beginning.....0.280 mg.)			
	No. of cells, after 23 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increases.	Determ.	Increases.
I	0	hrs.	—	mg.	mg.	mg.	mg.
II	215	—	—	0.280	0	0.280	0
III	736	—	—	0.280	0	0.595	0.315
IV	168×10^5	—	—	0.455	0.175	1.400	1.120
V	673×10^5	1.61	0.43	0.683	0.403	4.760	4.480
VI	172×10^6	1.41	0.49	1.050	0.770	6.890	6.615
VII	172×10^6	1.30	0.53	1.680	1.400	6.720	6.440
VIII	238×10^6	1.27	0.55	1.505	1.225	6.090	5.810
IX	189×10^6	1.29	0.54	1.715	1.435	6.213	5.933
XI	148×10^6	1.32	0.53	1.750	1.470	—	—
	146×10^6	1.32	0.53	1.610	1.330	5.600	5.320

Influence of CaCl_2 .—The rate of growth is slightly stimulated at 0.02–0.005 M, but decreased at higher concentration than that. The growth is inhibited at higher concentration than 0.1 M. The decomposition is stimulated at higher concentration than 0.001 M, and it is the most remarkable at 0.02–0.01 M but decreases at higher concentration than that.

Table 85. Influence of BaCl_2 .

No. of flask	Bacterial growth (inoculum, 836)			Volatile basic nitrogen (at the beginning.....0.280 mg.)			
	No. of cells, after 22 hrs.	<i>g</i>	<i>k</i>	After 5 days		After 20 days	
				Determ.	Increas.	Determ.	Increas.
		hrs.		mg.	mg.	mg.	mg.
I	0	—	—	0.280	0	0.280	0
II	0	—	—	0.280	0	0.280	0
III	0	—	—	0.280	0	0.280	0
IV	83	—	—	0.280	0	2.555	2.275
V	725	—	—	0.315	0.035	—	—
VI	918	—	—	0.350	0.070	4.655	4.375
VII	211×10	16.47	0.04	0.420	0.140	4.918	4.638
VIII	269×10^4	1.89	0.37	1.015	0.735	—	—
IX	183×10^5	1.53	0.45	—	—	4.918	4.638
XI	797×10^5	1.33	0.52	1.820	1.540	5.163	4.883

Influence of SrCl_2 :—The rate of growth is slightly stimulated at 0.005–0.0025 M and retarded at higher concentration than that. The growth is inhibited at higher concentration than 0.1 M. The decomposition is stimulated at about 0.01 M, but decreased at higher concentration and not influenced at lower concentration than that.

Influence of BaCl_2 :—This salt has a strong toxic action on bacteria. The growth is retarded at concentration of 0.001 M and inhibited at 0.01 M. Both the bacterial growth and the bacterial decomposition are never stimulated within the concentrations employed.

Influence of BeCl_2 :—This salt has very strong toxic action. The growth is inhibited even at 0.001 M. After 24 hours, the number of the organisms introduced in the inoculum is decreased to one-tenth at this concentration.

From the results obtained, the bacterial decomposition seems to be little influenced by alkali metals, but considerably influenced by alkali-earth metals. The decomposition seems to be influenced at lower concentration than the bacterial growth, with the same salt.

V. Influence of Heavy Metals.

The influence of metallic salts on the growth rate and on the death rate (used as disinfectants) of bacteria have been studied by various authors, but nothing is known of the relation between other physiological activity of bacteria, especially between bacterial decom-

position of nitrogenous compounds and metallic salts.

In the present study, the influence of metallic salts on the bacterial decomposition of fish muscle has been examined.

Experiment.

Metallic salts employed were as follows: AlCl_3 , MnCl_2 , FeCl_3 , NiCl_2 , CoCl_2 , CuCl_2 , ZnSO_4 , AgNO_3 , SnCl_2 , $\text{PtCl}_4 \cdot 2\text{HCl}$, $\text{AuCl}_3 \cdot \text{HCl}$ and HgCl_2 , and they were made in concentration of M/20. It may be mentioned that Cl' has comparatively weak action so that its effects should be nearly null at lower concentration than about 0.005 M. Therefore, the effects of the metallic salts of lower concentration than that, seems to be due to metallic ions.

The same method as that in Chapter III was used. And at regular intervals, amino and ammonium nitrogen in 5 c.c. of the mixtures was determined. Muscle suspension, solution of the metallic salts, bacterial suspension and sterilized distilled water were mixed with

Table 86. Composition of the decomposing mixtures.

No. of flask	I	II	III	IV	V	VI	VII	VIII	IX	X
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Muscle suspension	50	50	50	50	50	50	50	50	50	50
Solution of metallic salt	25	10	5	2	1	0.5	0.25	0.1	0.05	0
Distilled water	24	39	44	47	48	48.5	48.75	48.9	48.95	49
Bacterial suspension	1	1	1	1	1	1	1	1	1	1
Mol. concentration	0.0125	0.005	0.0025	0.001	0.0005	0.00025	0.000125	0.00005	0.000025	0

Table 87. Initial pH-values of the mixtures.

Added salt	I	II	III	IV	V	VI	VII	VIII	IX	X
AlCl_3	5.0	5.4	5.9	6.0	6.1	6.1	6.1	6.1	6.1	6.1
MnCl_2	5.8	5.9	6.0	6.0	6.1	6.1	6.1	6.1	6.1	6.1
FeCl_3	—	—	—	6.1	6.1	—	6.1	6.1	6.1	6.1
NiCl_2	5.4	5.6	5.9	6.0	6.1	6.1	6.1	6.1	6.1	6.1
CoCl_2	5.3	5.6	5.7	5.9	6.0	6.1	6.1	6.1	6.1	6.1
CuCl_2	5.5	5.7	5.8	5.9	6.1	6.1	6.1	6.1	6.1	6.1
ZnSO_4	5.3	5.6	5.8	6.0	6.1	6.1	6.1	6.1	6.1	6.1
AgNO_3	—	—	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
SnCl_2	—	5.7	5.9	6.0	6.1	6.1	6.1	6.1	6.1	6.1
$\text{PtCl}_4 \cdot 2\text{HCl}$	—	—	—	5.9	5.9	6.0	6.0	6.0	6.0	6.0
$\text{AuCl}_3 \cdot \text{HCl}$	—	—	—	5.9	6.0	6.0	6.0	6.0	6.0	6.0
HgCl_2	—	5.2	5.7	5.9	6.0	6.0	6.0	6.0	6.0	6.0

each other as shown in Table 86. The initial pH-values of these mixtures are given in Table 87.

Results.

Results obtained are shown in Tables 88-99. From the data Figs. 41-52 are drawn. K and t_1 calculated from these figures are given in Tables 100 and 101.

Influence of AlCl_3 :— K is independent of the concentration of this salt and has the same value within the range of the concentration at which the decomposition can take place. The value of t_1 becomes larger with increasing concentration. The value of A becomes larger with increasing concentration and is the largest at the concentration of 0.0025 M, but the decomposition can not develop at higher concentration than that. From these facts it may be understood that when AlCl_3 was added to fish muscle, the inductive period (i.e. the time required for beginning of the rapid decomposition) becomes longer, but the duration of the decomposition thereafter becomes longer with the increasing of the concentration until the decomposition becomes unable to take place, although the speed of the decomposition is independent of the concentrations, so that fish muscle is decomposed in greater amount with increasing concentration of AlCl_3 .

Influence of MnCl_2 :—Both K and t_1 are independent of the concentration excepting 0.0125 M which is the limiting concentration to allow decomposition. At 0.0125 M only, K is smaller and t_1 is larger than at the other concentrations. However, the value of A increases

Table 88. Influence of AlCl_3 .

Time of incubation	II			III			IV		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455	—	—	0.455	—	—	0.455	—	—
2	—	—	—	0.455	0	—	0.490	0.035	2.143
3	0.455	0	—	0.455	0	—	0.498	0.043	2.234
5	—	—	—	0.473	0.018	3.736	0.910	0.455	1.336
7	0.455	0	—	0.840	0.385	1.120	1.750	1.295	0.012
9	—	—	—	1.855	1.400	1.862	2.345	1.890	0.454
11	—	—	—	2.888	2.433	0.436	2.870	2.415	1.237
12	—	—	—	3.255	2.800	0.728	3.010	2.555	—
15	—	—	—	3.745	3.290	1.974	3.010	2.555	—
17	0.455	0	—	3.780	3.325	—	—	—	—

Table 88. continued.

Time of incubation	V			VI			VII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
2	0.490	0.035	2.208	0.455	0		0.498	0.043	2.278
3	0.543	0.088	2.620	0.578	0.123	2.787	0.683	0.228	1.038
5	1.120	0.665	1.636	1.243	0.788	1.768	1.313	0.858	1.771
7	2.065	1.610	0.433	2.170	1.715	0.612	2.223	1.768	0.514
9	2.555	2.100	1.301	2.520	2.065	1.471	2.625	2.170	1.191
11	2.625	2.170	1.793	—	—	—	2.765	2.310	
12	—	—	—	—	—	—	2.765	2.310	
15	2.660	2.205		2.590	2.135		2.765	2.310	
17	2.660	2.205		2.590	2.135				

Table 88. continued.

Time of incubation	VIII			IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
2	0.630	0.175	2.900	0.613	0.158	2.981	0.590	0.140	2.878
3	0.753	0.298	1.156	0.840	0.385	1.317	0.805	0.350	1.328
5	1.315	0.860	1.754	1.540	1.085	1.974	1.505	1.050	0.047
7	2.415	1.960	0.670	2.380	1.925	0.787	2.065	1.610	0.622
9	2.730	2.275	1.336	2.590	2.135	1.309	2.345	1.890	1.256
11	2.835	2.380		2.695	2.240		2.450	1.995	
12	2.835	2.380		2.695	2.240		2.450	1.995	
15				—	—		2.450	1.995	
17				2.695	2.240				

Table 89. Influence of MnCl_2 .

Time of incubation	I			II			III		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
2	—	—	—	0.560	0.105	2.366	0.560	0.105	2.341
4	0.508	0.053	2.085	1.295	0.840	1.347	1.470	1.015	1.418
6	0.805	0.350	2.937	3.010	2.555	0.085	3.115	2.660	0.075
7	1.225	0.770	1.326	3.990	3.535	0.514	4.585	4.130	0.730
8	1.785	1.330	1.636	4.725	4.270	1.087	5.040	4.585	1.163
9	2.170	1.715	1.804	4.900	4.445	1.405	5.355	4.900	
11	3.430	2.975	0.317	5.075	4.620		5.355	4.900	
13	4.305	3.850	0.838	5.075	4.620		5.355	4.900	
15	4.760	4.305	1.585	5.075	4.620				
18	4.865	4.410							

Table 89. continued.

Time of incubation	IV			V			VI		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
2	0.560	0.105	2.553	0.525	0.070	2.415	0.508	0.053	2.302
4	1.015	0.560	1.353	1.033	0.578	1.412	0.963	0.508	1.366
6	2.415	1.960	1.974	2.135	1.680	1.907	2.030	1.575	0.148
7	2.975	2.520	0.682	2.730	2.275	0.668	2.730	2.275	0.734
8	3.325	2.870	1.215	2.940	2.485	0.949	2.940	2.485	1.073
9	3.395	2.940	1.448	3.080	2.625	1.274	3.045	2.590	1.392
11	3.500	3.045		3.220	2.765		3.150	2.695	
13	3.500	3.045		3.220	2.765		3.150	2.695	
15				3.220	2.765				
18									

Table 89. continued.

Time of incubation	VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.508	0.053	2.326	0.490	0.035	2.222
4	0.980	0.525	1.413	0.945	0.490	1.475
6	1.838	1.383	0.072	1.785	1.330	0.218
7	2.450	1.995	0.562	2.275	1.820	0.762
8	2.695	2.240	0.853	2.450	1.995	0.154
9	2.905	2.450	1.369	3.520	2.065	1.471
11	3.010	2.555		3.590	2.135	
13	3.010	2.555		3.590	2.135	
15	3.010	2.555				
18						

Table 89. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.490	0.035	2.237	0.525	0.070	2.569
4	0.918	0.463	1.461	0.875	0.420	1.436
6	1.785	1.330	0.258	1.540	1.085	0.094
7	2.170	1.715	0.691	1.960	1.505	0.520
8	2.345	1.890	1.034	2.205	1.750	0.922
9	2.468	2.013	1.588	2.345	1.890	1.431
11	3.520	2.065		2.415	1.960	
13	3.520	2.065		2.415	1.960	
15				2.415	1.960	
18						

Table 90. Influence of FeCl_3 .

Time of incubation	IV			V			VII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.490			0.490			0.490		
2	0.665	0.175	2.781	0.665	0.175	2.770	0.665	0.175	2.781
4	1.418	0.928	1.635	1.663	1.173	1.774	1.453	0.963	1.659
6	2.590	2.100	0.332	2.853	2.363	0.478	2.800	2.310	0.478
8	3.255	2.765	0.945	3.535	3.045	1.464	3.430	2.940	1.323
10	3.500	3.010	1.634	3.640	3.150		3.570	3.080	
12	3.570	3.080		3.640	3.150		3.570	3.080	

Table 90. continued.

Time of incubation	VIII			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.490			0.490		
2	0.665	0.175	2.838	0.665	0.175	2.887
4	1.365	0.875	1.675	1.330	0.840	1.718
6	2.590	2.100	0.527	2.345	1.855	0.494
8	3.010	2.520	1.090	2.800	2.310	1.218
10	3.215	2.725		2.940	2.450	
12	3.215	2.725		2.940	2.450	

Table 91. Influence of NiCl_2 .

Time of incubation	IV			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	—	—	—	0.490	0.035	2.357
4	0.490	0.035	2.659	0.928	0.473	1.633
6	0.770	0.315	1.809	1.470	1.015	0.259
7	0.945	0.490	0.193	1.610	1.155	0.440
9	1.050	0.595	0.453	1.890	1.435	1.011
11	1.155	0.700	0.825	1.995	1.540	1.644
13	1.225	0.770	1.343	2.030	1.575	
15	1.260	0.805		2.030	1.575	

Table 91. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.498	0.043	2.376	0.525	0.070	2.569
4	0.910	0.455	1.513	0.875	0.420	1.436
6	1.470	1.015	0.082	1.540	1.085	0.094
7	1.785	1.330	0.404	1.960	1.505	0.520
9	2.240	1.785	1.407	2.345	1.890	1.432
11	2.310	1.855		2.415	1.960	
13	2.310	1.855		2.415	1.960	
15	2.310	1.855		2.415	1.960	

II, III.....the decomposition does not take place.

Table 92. Influence of CoCl_2 .

Time of incubation	VI			VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
2	0.455	0		0.525	0.070	2.839	0.543	0.088	2.672
4	0.560	0.105	1.046	0.770	0.315	1.612	0.945	0.490	1.524
6	0.770	0.315	1.633	1.033	0.578	0.057	1.750	1.295	0.290
8	1.050	0.595	0.117	1.400	0.945	0.830	2.240	1.785	1.009
10	1.190	0.735	0.368	1.470	1.015	1.162	2.380	1.925	1.741
13	1.400	0.945	0.955	1.540	1.085		2.415	1.960	
15	1.505	1.050		1.540	1.085		2.415	1.960	
18	1.505	1.050		1.540	1.085		2.415	1.960	

Table 92. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.543	0.088	2.672	0.525	0.070	2.645
4	0.963	0.508	1.544	0.875	0.420	1.436
6	1.680	1.225	0.222	1.540	1.085	0.094
8	2.275	1.820	1.114	2.205	1.750	0.922
10	2.380	1.925	1.741	2.380	1.925	1.741
13	2.415	1.960		2.415	1.960	
15	2.415	1.960		2.415	1.960	
18	2.415	1.960		2.415	1.960	

IV, V.....the decomposition does not take place.

Table 94. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.560	0.105	2.645	0.525	0.070	2.645
4	1.120	0.665	1.572	0.875	0.420	1.436
6	1.925	1.470	0.177	1.540	1.085	0.094
8	2.415	1.960	0.603	2.205	1.750	0.922
10	2.695	2.240	1.029	2.380	1.925	1.741
13	2.835	2.380	1.532	2.415	1.960	
15	2.905	2.450		2.415	1.960	
18	2.905	2.450		2.415	1.960	
19						

I.....the decomposition does not take place.

II.....the decomposition take place very slightly.

Table 95. Influence of AgNO₃.

Time of incubation	VI			VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.490			0.490			0.490		
2	—	—	—	—	—	—	0.595	0.105	2.700
4	—	—	—	0.648	0.158	2.919	0.980	0.490	1.456
6	0.648	0.158	2.919	1.050	0.560	1.571	1.820	1.330	0.182
8	1.155	0.665	1.677	1.855	1.365	0.290	2.520	2.030	1.065
10	1.908	1.418	0.341	2.398	1.908	1.085	2.695	2.205	
11	—	—	—	—	—	—	2.695	2.205	
12	—	—	—	2.555	2.065				
13	2.555	2.065		2.555	2.065				
14	2.555	2.065							

Table 95. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.490			0.490		
2	0.613	0.123	2.771	0.630	0.140	2.783
4	1.068	0.578	1.551	1.173	0.683	1.588
6	1.890	1.400	0.240	2.135	1.645	0.310
8	2.520	2.030	1.065	2.800	2.310	1.218
10	2.695	2.205		2.940	2.450	
11	2.695	2.205		2.940	2.450	
12				2.940	2.450	
13						
14						

IV V.....the decomposition does not take place.

Table 96. Influence of SnCl_2 .

Time of incubation	IV			V			VI		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.490	—	—	0.490	—	—	0.490	—	—
2	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	0.595	0.105	2.700
6	0.910	0.420	1.427	0.980	0.490	1.472	1.155	0.665	1.636
8	1.610	1.120	0.108	1.803	1.313	0.204	1.925	1.435	0.271
10	2.170	1.680	0.727	2.415	1.925	0.962	2.485	1.995	0.978
11	—	—	—	—	—	—	—	—	—
13	2.485	1.995	—	2.625	2.135	—	2.695	2.205	—
14	2.485	1.995	—	2.625	2.135	—	2.695	2.205	—

Table 96. continued.

Time of incubation	VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.490	—	—	0.490	—	—
2	0.490	0	—	0.490	0	—
4	0.700	0.210	1.016	0.823	0.333	1.243
6	1.260	0.770	1.720	1.523	1.033	1.934
8	2.065	1.575	0.374	2.310	1.820	0.637
10	2.555	2.065	1.062	2.625	2.135	1.309
11	—	—	—	—	—	—
13	2.730	2.240	—	2.730	2.240	—
14	2.730	2.240	—	2.730	2.240	—

Table 96. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.490	—	—	0.490	—	—
2	—	—	—	0.630	0.140	2.783
4	0.910	0.420	1.364	1.173	0.683	2.588
6	1.575	1.085	1.974	2.135	1.645	0.310
8	2.310	1.820	0.637	2.800	2.310	1.218
10	2.625	2.135	1.309	2.940	2.450	—
11	—	—	—	2.940	2.450	—
13	2.730	2.240	—	2.940	2.450	—
14	2.730	2.240	—	—	—	—

II. III.....the decomposition does not take place.

Table 97. Influence of $\text{PtCl}_4 \cdot 2\text{HCl}$.

Time of incubation	VI			VII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
3	0.455	0		0.595	0.140	1.158
5	0.648	0.193	1.430	0.875	0.420	1.850
7	0.945	0.490	0.067	1.173	0.718	0.384
9	1.225	0.770	0.741	1.365	0.910	0.949
11	1.330	0.875	1.398	1.435	0.980	1.447
13	1.365	0.910		1.470	1.015	
15	1.365	0.910		1.470	1.015	

Table 97. continued.

Time of incubation	VIII			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
3	0.578	0.125	1.076	0.630	0.175	1.194
5	0.928	0.473	1.842	1.033	0.578	1.907
7	1.313	0.858	0.461	1.435	0.980	0.494
9	1.488	1.033	0.919	1.628	1.173	0.984
11	1.593	1.138	1.827	1.715	1.260	1.556
13	1.610	1.155		1.750	1.295	
15	1.610	1.155		1.750	1.295	

IV, V.....the decomposition does not take place.

Table 98. Influence of $\text{AuCl}_3 \cdot \text{HCl}$.

Time of incubation	VI			VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.455			0.455			0.455		
3	0.455	0		0.595	0.140	1.158	0.560	0.105	1.063
5	0.648	0.193	1.409	0.910	0.455	1.911	0.893	0.438	1.881
7	0.910	0.455	1.968	1.155	0.700	0.348	1.208	0.753	0.459
9	1.190	0.735	0.544	1.348	0.893	0.964	1.330	0.875	0.797
11	1.330	0.875	1.098	1.435	0.980	1.447	1.435	0.980	1.447
13	1.400	0.945		1.470	1.015		1.470	1.015	
15	1.400	0.945		1.470	1.015		1.470	1.015	

Table 98. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
3	0.595	0.140	1.140	0.630	0.175	1.194
5	0.945	0.490	1.868	1.033	0.578	1.907
7	1.330	0.875	0.495	1.435	0.980	0.494
9	1.488	1.033	0.919	1.628	1.173	0.984
11	1.593	1.138	1.827	1.715	1.260	1.556
13	1.610	1.155		1.750	1.295	
15	1.610	1.155		1.750	1.295	

IV, V.....the decomposition does not take place.

Table 99. Influence of HgCl_2 .

Time of incubation	VII			VIII		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.455	0		0.578	0.123	2.771
3	0.455	0		0.788	0.333	1.250
5	0.718	0.263	1.132	1.470	1.015	1.932
7	1.365	0.910	1.847	2.310	1.855	0.725
9	1.925	1.470	0.301	2.590	2.135	1.485
11	2.450	1.995	0.978	2.660	2.205	
12	2.590	2.135	—	2.660	2.205	
15	2.660	2.205				

Table 99. continued.

Time of incubation	IX			X		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
day	mg.	mg.		mg.	mg.	
0	0.455			0.455		
2	0.665	0.210	1.030	0.595	0.140	2.878
3	0.753	0.298	1.202	0.805	0.350	1.328
5	1.365	0.910	1.859	1.505	1.050	0.047
7	2.275	1.820	0.717	2.065	1.610	0.622
9	2.520	2.065	1.294	2.345	1.890	1.256
11	2.625	2.170		2.450	1.995	
12	2.625	2.170		2.450	1.995	
15						

V, VI.....the decomposition does not take place.

Table 100. The value of K .

	I	II	III	IV	V	VI	VII	VIII	IX	X
AlCl_3	—	—	0.340	0.350	0.350	0.350	0.350	0.350	0.350	0.350
MnCl_2	0.275	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405
FeCl_3	—	—	—	0.340	0.405	—	0.405	0.405	—	0.405
NiCl_2	—	—	—	0.185	—	—	—	0.280	0.380	0.380
CoCl_2	—	—	—	—	—	0.200	0.290	0.375	0.375	0.375
CuCl_2	—	—	—	—	—	—	—	0.250	0.350	0.350
ZnSO_4	—	—	0.210	0.230	0.230	0.230	0.230	0.230	0.230	0.395
AgNO_3	—	—	—	—	—	0.350	0.350	0.375	0.375	0.375
SnCl_2	—	—	—	0.330	0.330	0.330	0.330	0.330	0.330	0.400
$\text{PtCl}_4 \cdot 2\text{HCl}$	—	—	—	—	—	0.350	0.350	0.350	—	0.350
$\text{AuCl}_3 \cdot \text{HCl}$	—	—	—	—	—	0.350	0.350	0.350	0.350	0.350
HgCl_2	—	—	—	—	—	—	0.305	0.360	0.360	0.360

Table 101. The value of t_1 .

	I	II	III	IV	V	VI	VII	VIII	IX	X
AlCl_3	—	—	9.50	7.45	5.50	5.50	5.50	5.50	4.90	4.90
MnCl_2	9.45	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55
FeCl_3	—	—	—	5.20	4.70	—	4.70	4.70	—	4.70
NiCl_2	—	—	—	6.30	—	—	—	5.30	5.45	5.45
CoCl_2	—	—	—	—	—	7.90	5.40	5.40	5.40	5.40
CuCl_2	—	—	—	—	—	—	—	7.00	5.65	5.05
ZnSO_4	—	—	10.10	7.55	6.10	6.10	6.10	6.10	6.10	5.65
AgNO_3	—	—	—	—	—	8.95	7.00	5.15	5.15	5.15
SnCl_2	—	—	—	7.80	6.95	6.95	6.95	6.05	6.05	5.00
$\text{PtCl}_4 \cdot 2\text{HCl}$	—	—	—	—	—	6.70	5.70	5.70	—	5.70
$\text{AuCl}_3 \cdot \text{HCl}$	—	—	—	—	—	7.10	5.60	5.60	5.60	5.60
HgCl_2	—	—	—	—	—	—	7.60	5.25	5.25	5.25

with increasing concentration and becomes the greatest at 0.0025 M, and then decreases at higher concentration than that, but is larger even at 0.0125 M than two times of the value in the control. Namely, when MnCl_2 is added to fish muscle, the amount of the decomposition becomes larger with the amount of this salt, within the limiting concentration to allow decomposition.

Influence of FeCl_3 :—The activity of this salt has the same tendency as that of MnCl_2 . Both K and t_1 are independent of the concentration below 0.0005 M. K is smaller and t_1 is larger at higher concentration than 0.001 M. However, A becomes larger with increasing concentration and becomes the greatest at 0.0005 M. Therefore, when

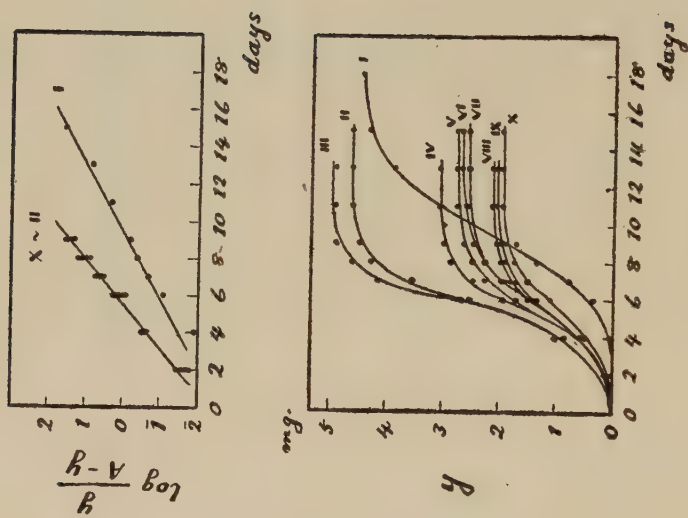


Fig. 42. Influence of MnCl_2 .

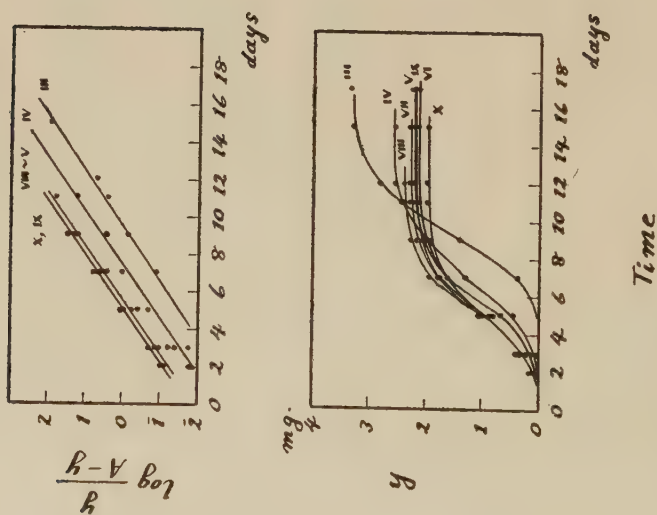


Fig. 41. Influence of AlCl_3 .

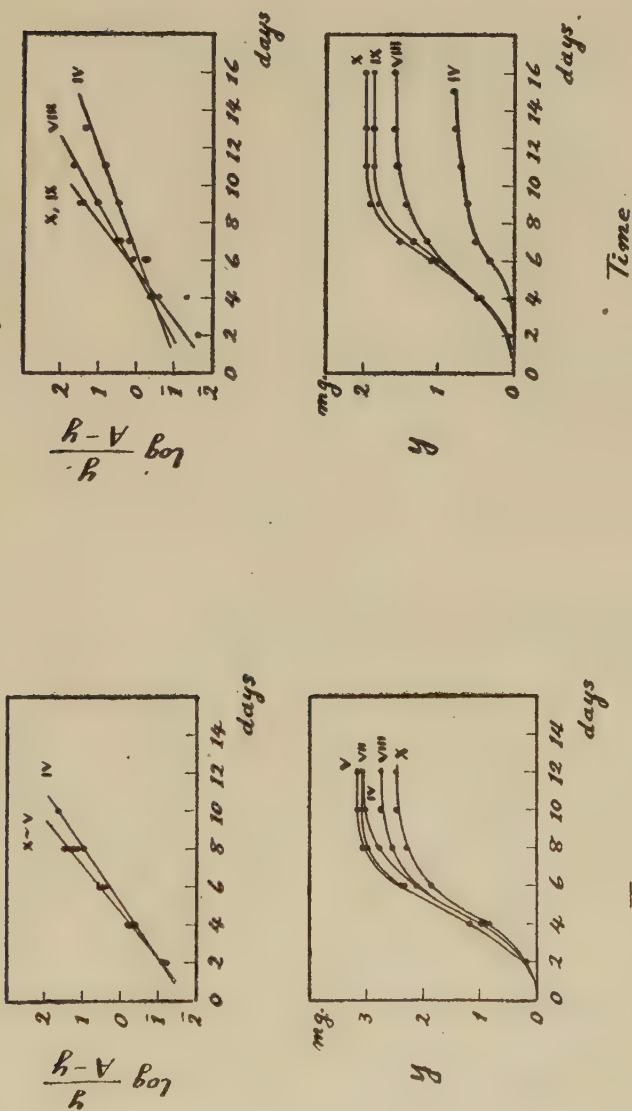
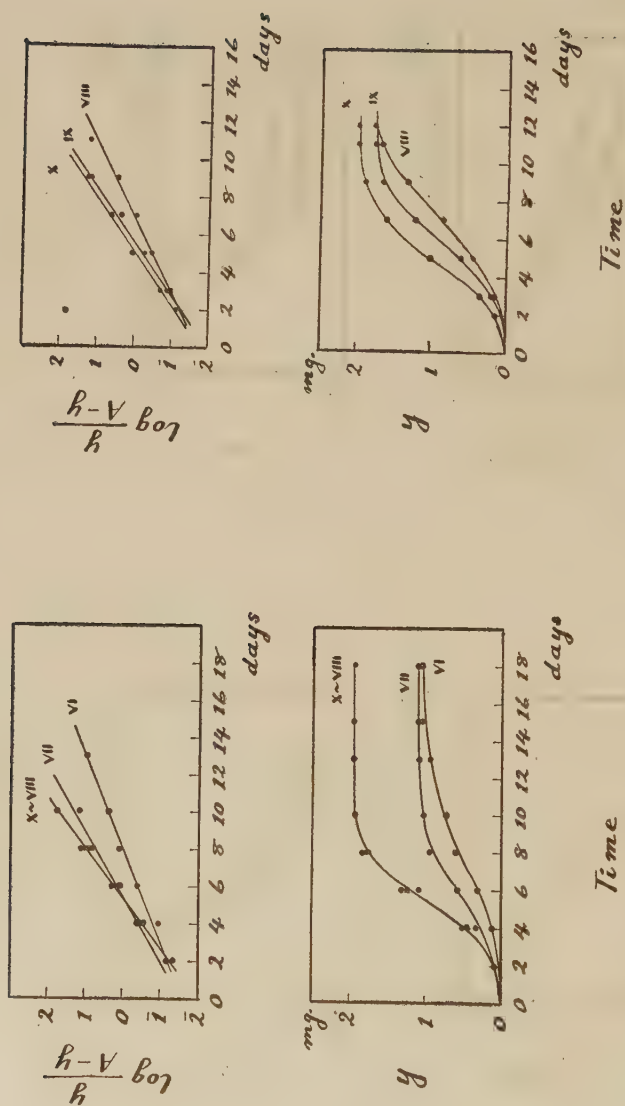


Fig. 44. Influence of NiCl₂.

Fig. 43. Influence of FeCl₃.

Fig. 46. Influence of CuCl_2 .Fig. 45. Influence of CoCl_2 .

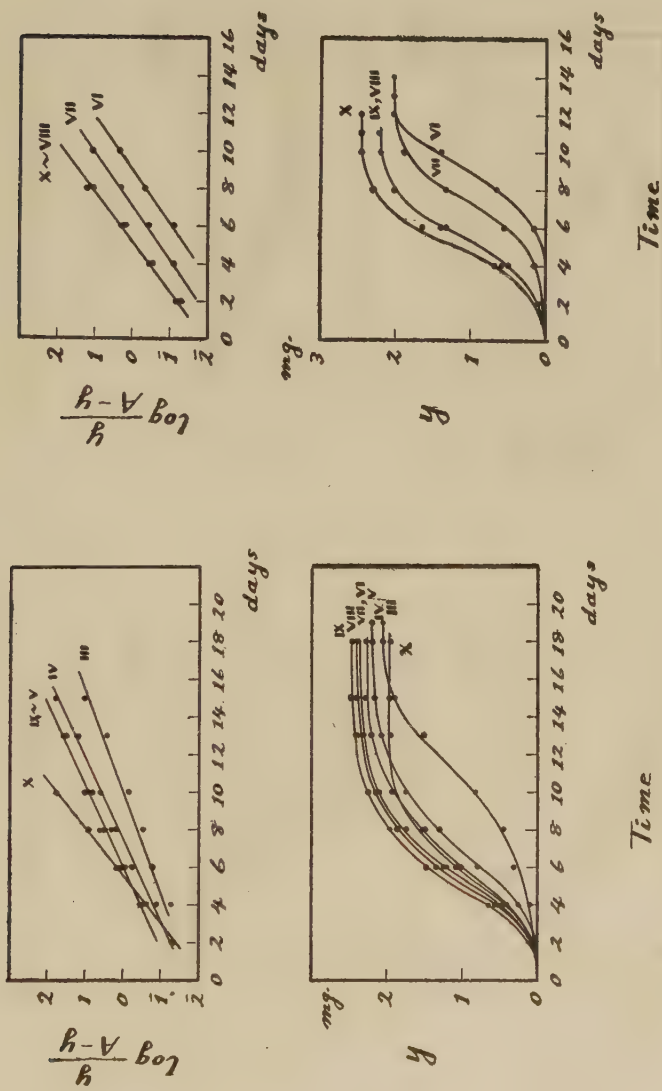
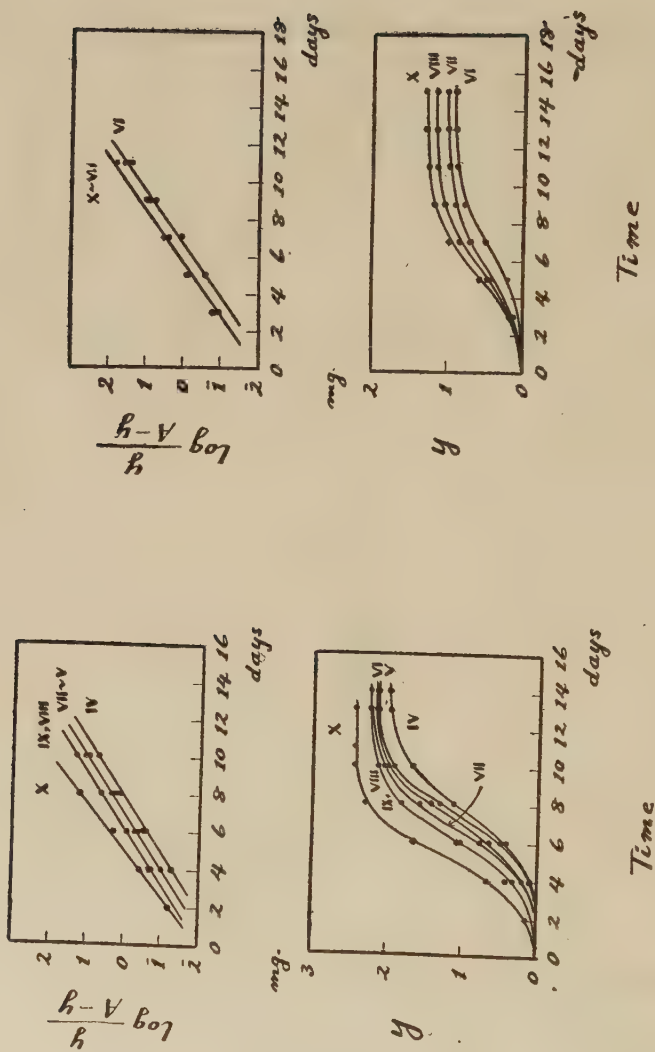


Fig. 48. Influence of AgNO_3 .

Fig. 47. Influence of ZnSO_4 .

Fig. 50. Influence of $\text{PtCl}_4 \cdot 2\text{HCl}$.Fig. 49. Influence of SnCl_2 .

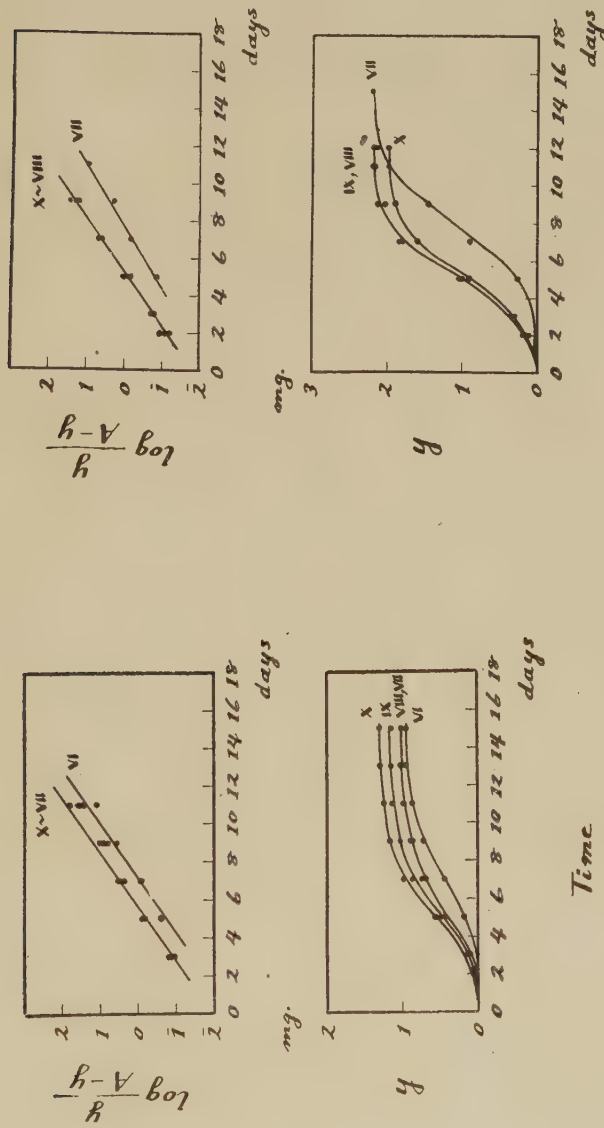


Fig. 51. Influence of AuCl₃·HCl.

Fig. 52. Influence of HgCl₂.

FeCl_3 is added to fish muscle the decomposition has the same tendency as in the case of MnCl_2 .

Influence of NiCl_2 .— K at 0.000025 M is the same as that in the control, but at higher concentration it decreases with increasing concentration. A is smaller even at 0.000025 M than in the control and becomes smaller with increasing concentration. Namely, the decomposition can not take place above 0.0025 M.

Influence of CoCl_2 .—This salt has the same tendency as NiCl_2 . K is the same at lower concentration than 0.00005 M, and decreases at higher concentration than 0.000125 M. And also A is smaller at higher concentration than 0.000125 M. The value of t_1 is greater at the concentration of 0.00025 M. Namely, injurious action is given to the decomposition by the addition of this salt.

Influence of CuCl_2 .—This salt has the same tendency as NiCl_2 and CoCl_2 . The value of K is the same at the concentration of 0.000025 M as in the control, but decreases at higher concentration than that. The value of A is decreased even at 0.000025 M and more decreased with increasing concentration. The value of t_1 becomes larger with increasing concentration. Namely, when CuCl_2 was added to fish muscle, it becomes to be hardly decomposed.

Influence of ZnSO_4 .—The effect of this salt is varied from the already mentioned salts. The value of K is remarkably smaller even at the concentration of 0.000025 M than in the control, but is unchanged by increasing concentration until they reach 0.001 M, and becomes more smaller at higher concentration than 0.0025 M. The value of t_1 is almost the same at lower concentration than 0.0005 M, and becomes larger at higher concentration than that. The value of A is the greatest at the concentration of 0.000025 M and decreases at higher concentration than that, but is larger even at 0.0025 M than in the control. The decomposition is not allowed to develop at higher concentration than 0.005 M.

Influence of AgNO_3 .—The value of K is the same at lower concentration than 0.00005 M and decreases at higher concentration than 0.000125 M. The value of t_1 is approximately the same at lower concentration than 0.00005 M but becomes larger at higher concentration than 0.000125 M. The value of A is retarded even at the concentration of 0.000025 M and decreased with increasing concentration. The decomposition does not take place at higher concentration than 0.0025 M. Fish muscle becomes to be hardly decomposed by the addition of this salt.

Influence of SnCl_2 :—The value of K is smaller even at 0.000025 M than in the control, but does not decrease with increasing concentration and is almost the same until they reach 0.001 M. The value of A becomes smaller and the value of t_1 becomes larger with increasing concentration. Therefore, when SnCl_2 is added to fish muscle, the amount of the decomposition decreases with the quantity of it.

Influence of $\text{PtCl}_4 \cdot 2\text{HCl}$:—The value of K is independent of the concentration. The value of t_1 is the same at lower concentration than 0.000125 M, but becomes larger at 0.00025 M. The value of A becomes smaller with increasing concentration. The decomposition does not take place at higher concentration than 0.0005 M. By the addition of this salt, the decomposition of fish muscle is retarded and the amount of the decomposition is decreased with increasing amount of this salt.

Influence of $\text{AuCl}_3 \cdot \text{HCl}$:—The effect of this salt is almost the same as that of $\text{PtCl}_4 \cdot 2\text{HCl}$.

Influence of HgCl_2 :—Both K and t_1 are approximately the same at lower concentration than 0.00005 M, but the former is small and the latter is large at the concentration of 0.000125 M. The value of A increases with increasing concentration. At higher concentration than 0.00025 M the decomposition does not take place. Although the effect of this salt is very weak and is significant only in the case of very low concentration, it seems to have tendency as MnCl_2 or FeCl_3 .

The results obtained can be summarized as follows:

The influence of various metallic salts on the bacterial decomposition of fish muscle varies with the kind of them. The value of A (showing the final rate of the decomposition) is either increased or decreased according to the kind and the concentration of metallic salts employed, but generally the value of K (showing the speed of the decomposition) is not increased by any one of the metallic salts employed but is unchanged or decreased. The effect of metallic salts upon the bacterial decomposition of fish muscle may be divided into three groups according to their kind, as follows: (1) AlCl_3 , MnCl_2 and FeCl_3 increase the value of A with increasing concentration of them up to a certain definite and comparatively high concentration but retard it markedly at higher concentration than that, whereas the value of K is almost unchanged, exclusive of very high concentration. (2) In the cases of NiCl_2 , CoCl_2 , CuCl_2 , SnCl_2 , AgNO_3 , $\text{PtCl}_4 \cdot 2\text{HCl}$ and $\text{AuCl}_3 \cdot \text{HCl}$, both K and A are not increased but decreased even at comparatively low concentration. (3) These do not belong to any one of the two former groups. ZnSO_4 increases the value of A within the certain

range of concentration, but decreases the value of K even at low concentration. Although, HgCl_2 resembles (1)-group, the degree of the effect is very weak and is significant at very low concentration.

VI. Influence of Hydrogen Ion Concentration.

Influence of hydrogen ion concentration upon the physiology of bacteria has been studied along two main courses, namely, on the growth rate of bacteria and on the other physiological activities of them. The first course has attracted much more attention of the bacteriologists than the second, but much is left to be desired in the technique. The second course mostly concerns with the influence of initial pH of the medium on the fermentation by yeast, lactic acid bacteria, *Bact. coli*, etc. There are not many investigations such as carried out by MEYERHOF⁽⁷²⁾ into the oxidation of ammonia and nitrite, or by ITANO⁽⁵⁹⁾ into the decomposition of broth media by *Bacillus subtilis* in relation to the aforementioned condition. Still fewer are the papers which have dealt with the decomposition of fish muscle under such condition.

The present study deals with the effect of initial pH upon the decomposition of fish muscle suspension as well as with the rate of growth of bacteria in it.

Experiment.

One hundred cubic centimeters of the fish muscle suspension (chub mackerel) prepared by the previously described method were put into a sterilized flask of the capacity of 300 c.c. and mixed with a certain volume of sterilized distilled water and of sterilized N/1 NaOH or N/1 HCl so as to fill 200 c.c. and to keep a definite hydrogen ion concentration.

Each flask is inoculated with bacteria and incubated at 26°C. After standing for a definite time, volatile basic nitrogen in 10 c.c., amino and ammonium nitrogen in 5 c.c. and bacterial counts in 1 c.c. were determined.

Organisms employed are as follows: *Pseudomonas fluorescens*, *Serratia marcescens*, *Achromobacter multistriatum*, *Proteus vulgaris* and *Staphylococcus aureus*.

Results.

Results obtained are shown in Tables 102-106. The generation time, g and the velocity coefficient of the growth rate, k were calculated from the already described equation.

Table 102. Influence of the initial hydrogen ion concentration on the bacterial growth and the decomposition of fish muscle (by *Pseudomonas fluorescens*).

pH	Bacterial growth (inoculum, 504)		k	Volatile basic nitrogen (at the beginning.....0.315 mg.)				Amino and ammonium nitrogen (at the beginning.....0.490 mg.)			
	No. of cells, after 22 hrs.	g		After 5 days		After 7 days		After 5 days		After 7 days	
				Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.
4.6	0	hrs.	—	mg. 0.315	mg. 0	mg. —	mg. —	mg. 0.490	mg. 0	mg. 0.490	mg. 0
5.5	121 × 10 ⁵	1.51	0.46	0.875	0.560	1.628	1.313	0.910	0.420	1.435	0.945
5.9	—	—	—	1.855	1.540	2.730	2.415	1.260	0.770	1.593	1.103
6.7	738 × 10 ⁵	1.28	0.54	1.470	1.155	1.610	1.295	1.015	0.525	1.050	0.560
7.0	484 × 10 ⁵	1.33	0.52	1.418	1.103	1.505	1.190	0.893	0.403	0.910	0.420
8.2	504 × 10 ⁵	1.32	0.53	1.225	0.910	1.260	0.945	0.700	0.210	0.735	0.425
9.1	384 × 10 ⁴	1.71	0.41	1.225	0.910	1.295	0.980	0.700	0.210	0.700	0.210

Table 103. Influence of the initial hydrogen ion concentration on the bacterial growth and the decomposition of fish muscle (by *Serratia marcescens*).

pH	Bacterial growth (inoculum, 831)		Volatile basic nitrogen (at the beginning.....0.315 mg.)								Amino and ammonium nitrogen (at the beginning.....0.490 mg.)			
	No. of cells, after 23 hrs.	g	k	After 5 days		After 7 days		After 5 days		After 7 days				
				Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.			
4.2	0	hrs.	—	mg. 0.315	mg. 0	mg. 0.315	mg. 0	mg. 0.490	mg. 0	mg. 0.490	mg. 0			
5.2	276×10^5	1.52	0.46	1.628	1.313	4.095	3.780	1.400	0.910	2.555	2.065			
5.9	342×10^6	1.23	0.56	2.328	2.013	5.040	4.725	1.820	1.330	3.150	2.660			
6.5	563×10^6	1.19	0.58	2.678	2.363	5.355	5.040	2.065	1.575	3.395	2.905			
7.5	310×10^6	1.24	0.56	2.380	2.065	4.375	4.060	1.995	1.505	2.905	2.415			
8.2	226×10^6	1.27	0.55	1.925	1.610	3.395	3.080	1.610	1.120	2.030	1.540			
9.2	180×10^6	1.30	0.53	1.680	1.365	2.590	2.275	1.260	0.770	1.820	1.330			

Table 104. Influence of the initial hydrogen ion concentration on the bacterial growth and the decomposition of fish muscle (by *Achromobacter multistriatum*).

pH	Bacterial growth (inoculum, 189)		k	Volatile basic nitrogen (at the beginning.....0.210 mg.)						Amino and ammonium nitrogen (at the beginning.....0.490 mg.)					
	No. of cells, after 20 hrs.	g		After 5 days		After 7 days		After 5 days		After 7 days					
				Determ.	Increases.	Determ.	Increases.	Determ.	Increases.	Determ.	Increases.				
4.7	23	—	—	mg. 0.223	mg. 0.013	mg. 0.223	mg. 0.013	mg. 0.490	mg. 0	mg. 0.503	mg. 0.013				
5.4	179 × 10 ⁴	1.54	0.46	0.350	0.140	0.753	0.543	0.560	0.070	0.770	0.280				
5.8	112 × 10 ⁵	1.28	0.54	0.350	0.140	0.753	0.543	0.560	0.070	0.805	0.315				
6.0	236 × 10 ⁵	1.18	0.59	0.473	0.263	—	—	0.630	0.140	0.840	0.350				
6.4	374 × 10 ⁵	1.14	0.61	1.155	0.945	1.260	1.050	0.945	0.455	1.050	0.560				
7.0	369 × 10 ⁵	1.14	0.61	—	—	1.260	1.050	0.980	0.490	1.050	0.560				
8.4	248 × 10 ⁵	1.18	0.59	1.120	0.910	1.383	1.173	0.998	0.508	1.120	0.630				
9.4	243	—	—	0.210	0	0.210	0	0.490	0	—	—				

Table 105. Influence of the initial hydrogen ion concentration on the bacterial growth and the decomposition of fish muscle (by *Proteus vulgaris*).

pH	Bacterial growth (inoculum, 621)			k.	Volatile basic nitrogen (at the beginning.....0.210 mg.)				Amino and ammonium nitrogen (at the beginning.....0.455 mg.)			
	No. of cells, after 20 hrs.	g	hrs.		After 5 days		After 7 days		After 5 days		After 7 days	
					Determ.	Increases.	mg.	mg.	Determ.	Increases.	mg.	Determ.
4.7	41	—	—	—	mg. 0.210	mg. 0	mg. 0.210	mg. 0.455	mg. 0.455	mg. 0	mg. 0	
5.4	562	—	—	—	—	0	0	0	0	0	0	0
5.8	618 × 10 ³	2.01	0.34	0.34	0.210	0	0.228	0.228	0.455	0.455	0	0.070
6.0	176 × 10 ⁴	1.74	0.40	0.40	0.473	0.263	0.350	0.350	0.455	0.455	0	0.140
6.4	388 × 10 ⁴	1.59	0.44	0.44	—	—	—	—	0.468	0.468	0.013	0.210
7.0	614 × 10 ⁴	1.51	0.46	0.46	0.473	0.263	0.333	0.333	0.508	0.508	0.053	0.263
8.4	724 × 10 ⁴	1.48	0.47	0.47	0.543	0.333	0.385	0.385	0.490	0.490	0.035	0.350
9.5	223 × 10 ³	2.30	0.29	0.29	0.385	0.175	0.373	0.373	—	—	—	0.315

Table 106. Influence of the initial hydrogen ion concentration on the bacterial growth and the decomposition of fish muscle (by *Staphylococcus aureus*).

pH	Bacterial growth (inoculum, 236×10^2)			Volatile basic nitrogen (at the beginning.....0.210 mg.)				Amino and ammonium nitrogen (at the beginning.....0.473 mg.)			
	No. of cells, after 20 hrs.	g	k	After 5 days		After 7 days		After 5 days		After 7 days	
				Determ.	Increases.	Determ.	Increases.	Determ.	Increases.		
4.7	416×10^3	hrs. 4.91	0.14	mg. 0.210	mg. 0	mg. 0.210	mg. 0	mg. 0.473	mg. 0	mg. 0.473	mg. 0
5.4	137×10^5	2.18	0.32	0.210	0	0.210	0	0.526	0.053	0.543	0.070
5.8	613×10^5	1.76	0.39	0.210	0	0.210	0	0.631	0.158	0.631	0.158
6.0	132×10^6	1.61	0.43	0.210	0	0.210	0	0.648	0.175	0.701	0.228
6.4	294×10^5	1.47	0.47	0.210	0	0.403	0.193	0.666	0.193	0.736	0.263
7.0	318×10^6	1.46	0.48	0.285	0.075	0.385	0.175	0.561	0.088	0.596	0.123
8.4	714×10^5	1.73	0.40	0.245	0.035	0.280	0.070	0.526	0.053	0.561	0.088
9.4	387×10^4	2.72	0.26	0.245	0.035	0.280	0.070	0.508	0.035	0.543	0.070

Various hydrogen ion concentrations may have markedly different influences upon the rate of bacterial growth, and upon the decomposition of fish muscle. Optimum hydrogen ion concentration of the rate of growth is more widely ranged than that of the decomposition of fish muscle.

Pseudomonas fluorescens:—The growth rate remains unchanged at about pH=6.0–8.0, and velocity coefficient k is about 0.53. But it decreases when hydrogen ion concentration of the medium shifts a little to acid or alkaline side, the decrease being more remarkable in the acid side than in alkaline. The decomposition of fish muscle is the greatest at about pH=6.0.

Serratia marcescens:—The growth rate reaches the maximum at about pH=6.5, and k is 0.58, but it decreases more steeply in acid side than in alkaline one. The decomposition is the greatest at about pH=6.5.

Achromobacter multistriatum:—The growth rate does not change so much within the range of pH-values from 6.0 to 8.0, but its peak appears to lie at about pH=6.5. The decomposition is nearly the same when pH-values ranges 6.5–8.5, but it suddenly falls down in the acid reaction.

Proteus vulgaris:—The growth rate seems to be the greatest at about pH=7.0–8.4. The decomposition appears to be the greatest at about pH=8.5.

Staphylococcus aureus:—It has a maximum in the growth rate at about pH=7.0. The decomposition seems to reach its culmination at about pH=6.5, although decomposing activity of the fish muscle by this organism is generally very weak.

VII. Influence of Carbohydrates.

It has long been known that fermentable carbohydrates retard bacterial proteolysis. There has developed an extensive literature on this so-called protein sparing effect of carbohydrates. According to KENDALL⁽⁶²⁾ and DEBORI⁽²³⁾ dextrose has a marked influence upon the nitrogenous metabolism of bacteria, that is bacterial proteolysis is retarded and the production of ammonia is greatly lessened by the presence of dextrose, and also degree of the effect becomes larger with increasing concentration of the sugar. Inhibition of ammonia formation from protein by *Bacterium alcaligenes* in the presence of sugar was observed by BOEHNCKE.⁽¹³⁾ One percent sugar decreased the production of ammonia to one-third. AUBEL and COLIN⁽⁴⁾ found that in the soil the process of ammonification is prevented by sugar. According to JONES,⁽⁶¹⁾

KENDALL and KEITH,⁽⁶⁴⁾ the inhibition of proteolysis by bacteria in media containing sugar is due to the absence of the enzyme. According to LEHMANN, WHERRY and GLENN,⁽³⁸⁾ since proteolytic enzymes of bacteria are active only in alkaline media, the production of acids from the sugar made the medium unfavorable for action. AUERBACH⁽⁶⁵⁾ formerly showed, however, that in a medium buffered by magnesium oxide sugar prevented the bacterial proteolysis. KENDALL, DAY and WALKER⁽⁶³⁾ concluded that it is due to the fact that the sugars better serve the energy needs of the organism. More recently, WESTENBERG⁽¹¹⁵⁾ found that in the case of various native methods of salting fish, if carbohydrates are added to the pickling brine in various forms, less salt is required. According to him, the preserving activity produced by the addition of carbohydrates is due not only to the grade of acidity but to the specific influence of the organic decomposition products.

The present study deals with the effect of various carbohydrates upon the progress of bacterial decomposition of fish muscle.

Experiment.

One hundred cubic centimeters of fish muscle suspension made by previously described method were put into a sterilized flask of a capacity of 300 c.c. and added with an equal volume of sterilized 2% solution of carbohydrate and inoculated with bacteria and then incubated at 26°C. At regular intervals, pH-values were determined and also total acidities were determined by reading off the quantity of N/40 NaOH as needed to neutralize 5 c.c. of the mixture, phenolphthalein being used as the indicator. Moreover, the determination of amino and ammonium nitrogen in 5 c.c. and of volatile basic nitrogen in 10 c.c. as well as bacterial counts in 1 c.c. was made.

Organisms employed were as follows: *Pseudomonas fluorescens*, *Serratia marcescens* and *Achromobacter tabificum*.

Carbohydrates used were as follows: Monosaccharides; arabinose, glucose, levulose, galactose. Disaccharides; lactose, maltose, sucrose. Trisaccharides; raffinose.

Results.

Results obtained are listed in Tables 107-109 and these data are shown in Figs. 53-55.

Table 107. Influence of carbohydrates on the bacterial growth and the decomposition of fish muscle (by *Pseudomonas fluorescens*).

a. Influence of arabinose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	289×10^5	—	—	—	—	—	5.80
2	117×10^7	—	—	—	—	—	5.65
3	219×10^7	0.210	0	0.560	0.070	2.10	5.55
5	—	0.210	0	0.560	0.070	2.10	5.45
7	358×10^7	0.223	0.018	0.578	0.088	2.00	5.40
10	—	0.473	0.263	0.875	0.385	2.00	5.90
12	—	1.365	1.155	1.435	0.945	1.90	6.60
15	—	4.235	4.025	2.380	1.890	1.60	7.45
18	—	5.180	4.970	2.625	2.135	0.50	8.00

Table 107. continued. b. Influence of glucose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	344×10^5	—	—	—	—	—	5.65
2	648×10^5	—	—	—	—	—	5.05
3	321×10^5	0.210	0	0.490	0	3.40	4.65
5	—	0.210	0	0.490	0	3.50	4.50
7	334×10^5	0.210	0	0.490	0	3.55	4.35
10	—	0.210	0	0.490	0	3.60	4.40
12	—	0.210	0	0.490	0	3.55	4.40
15	—	0.210	0	0.490	0	3.70	4.40

Table 107. continued. c. Influence of levulose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	101×10^6	—	—	—	—	—	—
2	139×10^7	—	—	—	—	—	6.05
3	218×10^7	0.543	0.333	0.735	0.245	1.60	6.30
5	—	0.578	0.368	0.945	0.455	2.25	6.20
7	661×10^6	0.570	0.360	0.893	0.403	3.70	4.50
10	—	0.570	0.360	0.893	0.403	4.30	4.50
12	—	0.570	0.360	0.893	0.403	4.35	4.50
15	—	0.570	0.360	0.875	0.385	4.40	4.50

Table 107. continued. d. Influence of galactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	362×10^5	—	—	—	—	—	5.60
2	768×10^6	—	—	—	—	—	5.35
3	328×10^6	0.210	0	0.490	0	2.75	5.15
5	—	0.210	0	0.490	0	2.60	5.00
7	516×10^6	0.210	0	0.490	0	2.35	4.85
10	—	0.210	0	—	—	1.75	4.90
12	—	0.210	0	0.490	0	1.75	5.20
15	—	0.210	0	0.490	0	1.30	5.20

Table 107. continued. e. Influence of lactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	304×10^5	—	—	—	—	—	—
2	115×10^7	—	—	—	—	—	6.10
3	241×10^7	0.735	0.525	0.718	0.228	1.50	6.40
5	—	1.505	1.295	1.470	0.980	1.50	6.90
7	175×10^7	2.730	2.520	2.100	1.610	1.15	7.40
10	—	4.270	4.060	2.765	2.275	—	7.70
12	—	5.040	4.830	2.975	2.485	0.30	8.20
15	—	5.163	4.953	2.975	2.485	0	8.60

Table 107. continued. f. Influence of maltose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	691×10^5	—	—	—	—	—	5.65
2	117×10^7	—	—	—	—	—	6.15
3	223×10^7	0.630	0.420	0.893	0.403	1.80	6.40
5	—	2.135	1.925	1.820	1.330	1.70	7.10
7	986×10^6	3.465	3.255	2.695	2.205	1.40	7.20
10	—	3.220	3.010	2.590	2.100	1.60	7.40
12	—	—	—	2.275	1.785	1.80	7.40
15	—	2.870	2.660	1.820	1.330	1.65	7.50

Table 107. continued. g. Influence of sucrose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	461 × 10 ⁵	—	—	—	—	—	5.90
2	119 × 10 ⁷	—	—	—	—	—	6.10
3	218 × 10 ⁷	0.595	0.385	0.718	0.228	1.55	6.30
5	—	1.908	1.698	1.488	0.998	1.55	7.00
7	239 × 10 ⁷	—	—	2.275	1.785	1.20	7.50
10	—	4.060	3.850	2.800	2.310	0.50	7.90
12	—	4.760	4.550	3.028	2.538	0	8.40
15	—	4.760	4.550	3.045	2.555	0	8.55

Table 107. continued. h. Influence of raffinose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	601 × 10 ⁵	—	—	—	—	—	—
2	116 × 10 ⁷	—	—	—	—	—	6.20
3	237 × 10 ⁷	0.595	0.385	0.840	0.350	1.55	6.40
5	—	1.943	1.733	1.645	1.150	1.50	7.10
7	965 × 10 ⁶	3.080	2.870	2.380	1.890	1.10	7.55
10	—	4.655	4.445	2.940	2.450	0.50	7.95
12	—	5.408	5.198	2.940	2.450	0	8.40
15	—	—	—	2.940	2.450	0	8.55

Table 107. continued. i. Control.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	694	0.210	—	0.490	—	2.05	5.80
22(hrs.)	591 × 10 ⁵	—	—	—	—	—	5.80
2	113 × 10 ⁷	—	—	—	—	—	6.15
3	153 × 10 ⁷	0.630	0.420	0.718	0.228	1.50	6.35
5	—	1.995	1.785	1.540	1.050	1.50	7.00
7	164 × 10 ⁷	2.940	2.730	2.310	1.820	1.15	7.45
10	—	4.725	4.515	3.045	2.555	0.50	7.95
12	—	—	—	3.156	2.666	0	8.40
15	—	6.300	6.090	3.156	2.666	0	8.55
18	—	6.300	6.090	—	—	—	—

Table 108. Influence of carbohydrates on the bacterial growth and the decomposition of fish muscle (by *Serratia marcescens*).

a. Influence of arabinose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	536×10^5	—	—	—	—	—	5.75
2	412×10^6	—	—	—	—	—	5.80
3	784×10^6	0.315	0.105	0.735	0.245	2.50	—
5	—	0.745	0.535	1.680	1.190	2.35	6.05
7	304×10^7	2.765	2.555	3.045	2.555	—	6.25
9	—	—	—	3.060	3.570	1.50	7.00
17	—	—	—	5.145	4.655	0	8.50

Table 108. continued. b. Influence of glucose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	411×10^5	—	—	—	—	—	5.60
2	381×10^6	—	—	—	—	—	5.40
3	321×10^6	0.210	0	0.700	0.210	3.75	5.30
5	—	0.228	0.018	0.753	0.263	4.30	5.20
7	348×10^6	0.210	0	0.893	0.403	4.85	—
9	—	0.210	0	0.945	0.455	5.30	4.80
17	—	0.245	0.035	1.485	0.595	6.60	4.60

Table 108. continued. c. Influence of levulose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	723×10^5	—	—	—	—	—	5.60
2	292×10^6	—	—	—	—	—	5.40
3	281×10^6	0.210	0	0.630	0.140	3.70	5.40
5	—	0.263	0.053	0.823	0.333	4.15	5.30
7	128×10^7	0.315	0.105	0.928	0.438	4.60	—
9	—	0.385	0.175	1.085	0.595	5.20	5.20
17	—	—	—	3.745	3.255	3.45	6.40

Table 108. continued. d. Influence of galactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	571×10^5	—	—	—	—	—	5.70
2	294×10^6	—	—	—	—	—	5.70
3	371×10^6	0.228	0.018	0.700	0.210	3.00	5.60
5	—	0.473	0.263	1.120	0.630	3.25	5.60
7	324×10^7	1.435	1.225	1.785	1.295	3.15	—
9	—	1.978	1.768	2.030	1.540	3.50	5.60
17	—	—	—	3.220	2.730	4.80	5.80

Table 108. continued. e. Influence of lactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	698×10^5	—	—	—	—	—	5.65
2	216×10^6	—	—	—	—	—	5.70
3	499×10^6	0.315	0.105	0.735	0.245	2.60	6.00
5	—	0.630	0.420	1.260	0.770	2.85	6.25
7	493×10^7	2.415	2.205	2.240	1.750	2.70	6.45
9	—	3.745	3.535	3.395	2.905	2.80	6.75
17	—	—	—	5.040	4.550	5.50	6.30

Table 108. continued. f. Influence of maltose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	848×10^5	—	—	—	—	—	5.70
2	288×10^6	—	—	—	—	—	5.45
3	275×10^6	0.210	0	0.630	0.140	3.30	5.40
5	—	0.298	0.088	0.840	0.350	3.85	5.35
7	121×10^7	0.490	0.280	1.015	0.525	4.45	—
9	—	0.490	0.280	1.155	0.665	4.70	5.30
17	—	—	—	2.625	2.135	4.00	5.60

Table 108. continued. g. Influence of sucrose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	119×10^6	—	—	—	—	—	5.60
2	421×10^6	—	—	—	—	—	5.35
3	548×10^6	0.210	0	0.630	0.140	3.80	5.35
5	—	0.228	0.018	0.735	0.245	4.00	5.20
7	131×10^7	0.245	0.035	0.945	0.455	4.75	—
9	—	0.333	0.123	1.085	0.595	4.90	5.20
17	—	—	—	3.290	2.800	2.10	6.40

Table 108. continued. h. Influence of raffinose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	771×10^6	—	—	—	—	—	5.80
2	401×10^6	—	—	—	—	—	5.80
3	698×10^6	0.315	0.105	0.700	0.210	2.50	6.10
5	—	1.103	0.893	1.540	1.050	2.50	6.45
7	388×10^7	2.695	2.485	3.010	2.520	1.90	6.80
9	—	4.848	4.638	4.270	3.780	1.60	7.00
17	—	—	—	6.160	5.670	0	8.50

Table 108. continued. i. Control.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	998	0.210	—	0.490	—	1.90	5.80
20(hrs.)	582×10^5	—	—	—	—	—	5.70
2	408×10^6	—	—	—	—	—	5.75
3	584×10^6	0.315	0.105	0.718	0.228	2.65	6.10
5	—	0.735	0.525	1.400	0.910	2.50	6.30
7	138×10^7	2.765	2.555	2.818	2.328	2.40	6.75
9	—	4.725	4.515	4.200	3.710	1.50	6.85
17	—	—	—	6.125	5.635	0	8.50

Table 109. Influence of carbohydrates on the bacterial growth and the decomposition of fish muscle (by *Achromobacter tabificum*).

a. Influence of glucose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	821×10^5	—	—	—	—	—	—
2	582×10^6	—	—	—	—	—	5.25
3	412×10^6	0.263	0.053	0.630	0.140	3.60	5.20
5	242×10^6	0.263	0.053	0.735	0.245	4.40	5.00
7	—	0.420	0.210	0.805	0.315	4.30	5.00
9	642×10^6	0.455	0.245	0.910	0.420	4.40	5.00
12	—	0.508	0.298	0.998	0.508	4.40	5.00
15	—	0.508	0.298	1.155	0.665	4.40	5.00

Table 109. continued. b. Influence of levulose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	973×10^5	—	—	—	—	—	—
2	803×10^6	—	—	—	—	—	5.40
3	442×10^6	0.315	0.105	0.665	0.175	3.90	5.20
5	446×10^6	—	—	0.735	0.245	4.20	5.00
7	—	0.385	0.175	0.788	0.298	4.00	5.00
9	611×10^6	0.385	0.175	0.963	0.473	4.20	5.00
12	—	0.770	0.560	1.155	0.665	4.30	5.20
15	—	0.770	0.560	1.295	0.805	4.50	5.20

Table 109. continued. c. Influence of galactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	486×10^5	—	—	—	—	—	—
2	776×10^6	—	—	—	—	—	5.50
3	768×10^6	0.315	0.105	0.700	0.210	3.90	5.40
5	753×10^6	—	—	0.770	0.280	4.30	5.20
7	—	0.438	0.228	0.840	0.350	4.25	5.05
9	—	0.473	0.263	0.945	0.455	4.30	5.15
12	—	0.578	0.368	1.120	0.630	4.50	5.10
15	—	—	—	1.155	0.665	4.30	5.10

Table 109. continued. d. Influence of lactose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	112×10^6	—	—	—	—	—	—
2	482×10^6	—	—	—	—	—	5.85
3	102×10^7	0.368	0.158	0.665	0.175	2.00	6.00
5	208×10^7	0.630	0.420	1.015	0.525	2.20	6.25
7	—	1.470	1.260	1.575	1.085	2.30	6.40
9	304×10^7	2.223	2.013	2.135	1.645	3.00	6.40
12	—	2.835	2.625	2.380	1.890	3.30	6.40
15	—	—	—	2.625	2.135	3.90	6.40

Table 109. continued. e. Influence of sucrose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	985×10^5	—	—	—	—	—	—
2	203×10^6	—	—	—	—	—	5.25
3	141×10^6	0.210	0	0.525	0.035	3.50	5.20
5	198×10^6	0.210	0	0.560	0.070	3.60	5.00
7	—	0.228	0.018	0.613	0.123	3.60	5.00
9	568×10^6	0.245	0.035	0.700	0.210	3.75	5.00
12	—	0.245	0.035	0.770	0.280	3.70	5.00
15	—	—	—	0.875	0.385	3.70	5.00

Table 109. continued. f. Influence of raffinose.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increases.	Determ.	Increases.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	560×10^5	—	—	—	—	—	—
2	135×10^6	—	—	—	—	—	5.50
3	183×10^6	0.210	0	0.560	0.070	3.10	5.20
5	512×10^6	0.263	0.053	0.770	0.280	3.50	5.40
7	—	0.455	0.245	0.945	0.455	3.80	5.40
9	198×10^7	0.805	0.595	1.190	0.700	4.30	5.40
12	—	1.628	1.418	1.330	0.840	4.30	5.40
15	—	—	—	1.505	1.015	4.30	5.50

Table 109. continued. g. Control.

Time of incubation	No. of cells	Volatile bas. N		Amino a. ammon. N		Total acidity	pH
		Determ.	Increas.	Determ.	Increas.		
days		mg.	mg.	mg.	mg.	c.c.	
0	447	0.210	—	0.490	—	2.10	5.80
20(hrs.)	498×10^6	—	—	—	—	—	—
2	169×10^6	—	—	—	—	—	5.85
3	343×10^6	0.350	0.140	0.665	0.175	2.10	6.00
5	987×10^6	0.560	0.350	0.823	0.333	1.95	6.25
7	—	1.295	1.085	1.470	0.980	2.00	6.45
9	998×10^6	2.153	1.943	2.415	1.925	2.00	6.45
12	—	2.975	2.765	2.870	2.380	1.80	7.25
15	—	3.045	2.835	3.255	2.765	0.80	7.65

The bacterial decomposition of fish muscle is not almost stimulated by the addition of carbohydrates, but is unchanged or decreased. This decrease is accompanied by the bacterial fermentation of carbohydrates in the medium. Hexose is generally most easily fermented by microorganisms. Though not clear, inhibiting action of these carbohydrates on the bacterial decomposition of fish muscle is probably due not only to the change of pH and of total acidity but also to the acid molecules produced and other fermentation products. The effect of carbohydrates on the growth rates of organisms is almost negligibly small, although the final amount of living cells is slightly influenced by them.

Pseudomonas fluorescens.:—Hexose has the greatest inhibitory power for the bacterial decomposition of fish muscle. Volatile base and amino and ammonium nitrogen are not formed in the medium containing glucose or galactose. In either case the pH-value of media tends to become acidic, although that of glucose becomes smaller than that of galactose. Total acidity of the former increases with decreasing pH-value, and that of the latter increases at first but decreasing gradually afterwards. The effect of levulose on the decomposition differs from that of glucose and galactose, viz., action of proteolysis is nearly the same as that of the control during the first 3–4 days, but from this time the formation of volatile base and of amino and ammonium nitrogen is suddenly ceased, pH-value becoming smaller and total acidity larger. Arabinose is one of monosaccharides, but its action differs from that of the above mentioned three, forming volatile base and amino and ammonium nitrogen very slightly, with pH-value

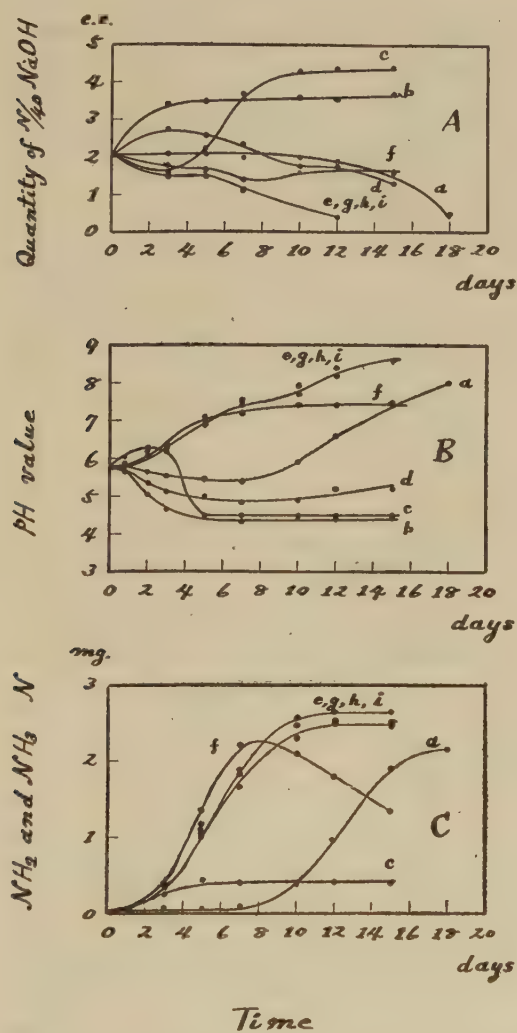


Fig. 53. Influence of carbohydrates on the bacterial decomposition of fish muscle (by the action of *Pseudomonas fluorescens*).

A...total acidity, B...pH-value, C...amino and ammonium N.
a...arabinose, b...glucose, c...levulose, d...galactose, e...lactose,
f...maltose, g...sucrose, h...raffinose, i...control.

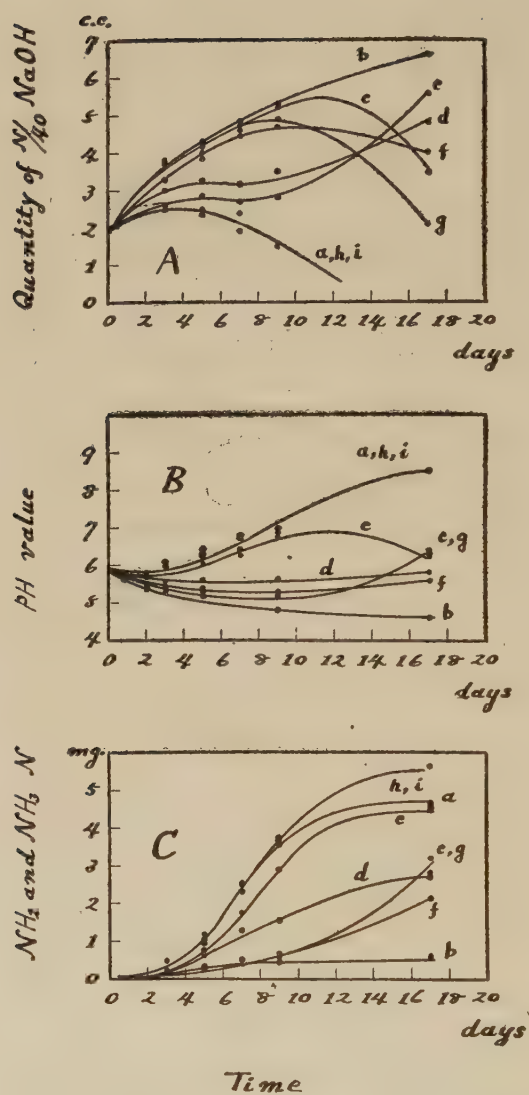


Fig. 54. Influence of carbohydrates on the bacterial decomposition of fish muscle (by the action of *Serratia marcescens*).

A, B, C and a, b, c, etc. are the same in Fig. 53.

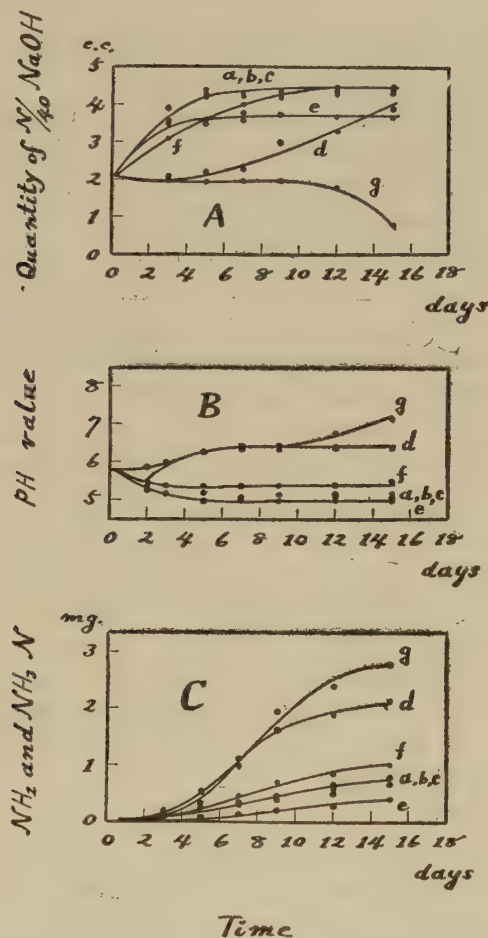


Fig. 55. Influence of carbohydrates on the bacterial decomposition of fish muscle (by the action of *Achromobacter tabificum*).

A, B and C are the same in Fig. 53.

a...glucose, b...levulose, c...galactose, d...lactose, e...sucrose, f...raffinose, g...control.

slightly smaller, and with total acidity almost unchanged during the first 8-9 days. Soon after this time pH-value becomes larger, total acidity becomes smaller, and the decomposition simultaneously take place. The bacterial decomposition is not affected by maltose during the first period, but from about 7 days it is suddenly ceased. Until about 7 days, pH-value of the maltose media progresses to alkaline side just as the control does, but afterwards it almost ceases to change in spite of ever increasing alkaline tendency of the control. Total acidity slightly decreases. The decomposition of fish muscle seems

to be hardly influenced by lactose, sucrose and raffinose, although it seems to be slightly prevented by lactose.

Effect of carbohydrates employed on the rate of the growth of bacteria is nearly null, but that on the final amount of living cells is little larger, smallest when glucose is present.

Serratia marcescens.—It seems that the decomposition of fish muscle is not influenced by raffinose and arabinose. By lactose the decomposition is slightly decreased, pH-value gradually turning to alkaline until about 9 days and then to acid side. Total acidity becomes larger until about 5 days with its suspension until about 9 days when it becomes larger again. Glucose, levulose, sucrose and maltose inflict the same effect on the decomposition for 7–8 days, increasing quantity of the formation of volatile base and of amino and ammonium nitrogen very slightly, making pH-value smaller and total acidity larger. In the case of glucose, after this period the decomposition is ceased and pH-value becomes more smaller and total acidity becomes more larger. But in the cases of other three, the decomposition is much more progressed. Effect of galactose on the decomposition is intermediate between that of lactose and of the above mentioned four, unchanging pH-value approximately, and having trend of change of total acidity nearly the same as that of lactose, with its value slightly larger.

Achromobacter tabificum.—The decomposition of fish muscle by this germ was retarded by the following carbohydrates arranged in descending order: sucrose > glucose > galactose > levulose > raffinose > lactose. It may be mentioned that glucose, galactose and levulose differ very hardly from each other in the effect under consideration. The formation of volatile base and of amino and ammonium nitrogen is more inhibited by sucrose than by the above mentioned three, but total acidity is rather smaller and pH-value is nearly the same.

VIII. Influence of Various Organic Acids.

As for the bactericidal action of various acids, studies hitherto reported show that the action is principally due to the hydrogen ion and is generally, with exceptions with certain acids, not very much due to the undissociated molecules or to the anions.⁽¹¹⁰⁾ So far as the present author is aware, however, little is known of the effects of acids on the physiological activity of bacteria, such as to decompose nitrogenous compounds.

In order to preserve the meat of aquatic animals such as fish and shellfish or to manufacture various products from it, the material is often treated with various acids. And also, in order to prevent it from the bacterial decomposition by fermenting products such as various acids, sugars are added into pickles. In these cases, if the solution is acidic with high hydrogen ion concentration, bacteria do not develop, so that it is reasonable that the decomposition does not occur. However, the decomposition is arrested often even at low hydrogen ion concentration.

The present study aims to ascertain how the effect is inflicted upon the bacterial decomposition by various acids irrespective of hydrogen ion concentration.

Experiment.

Fifty cubic centimeters of the solution of meat extract (chub mackerel) prepared by the already described method were put into a flask of a capacity of 200 c.c., and mixed with 50 c.c. of the solution of organic acid salt in varied concentrations, and then sterilized. Each flask was inoculated with *Pseudomonas fluorescens* and incubated at 26°C. At regular intervals, amino and ammonium nitrogen in 5 c.c. of the mixtures was determined.

In order to avoid the effect of hydrogen ion concentration, organic acids employed were previously made with Na-salt and prepared as the solution in varied concentrations.

Monobasic fatty acids: formic acid $\text{H} \cdot \text{COOH}$, acetic acid $\text{CH}_3 \cdot \text{COOH}$, propionic acid $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}$, butyric acid $\text{CH}_3 \cdot (\text{CH}_2)_2 \cdot \text{COOH}$, caproic acid $\text{CH}_3 \cdot (\text{CH}_2)_4 \cdot \text{COOH}$, nonylic acid $\text{CH}_3 \cdot (\text{CH}_2)_7 \cdot \text{COOH}$, lauric acid $\text{CH}_3 \cdot (\text{CH}_2)_{10} \cdot \text{COOH}$, myristic acid $\text{CH}_3 \cdot (\text{CH}_2)_{12} \cdot \text{COOH}$, palmitic acid $\text{CH}_3 \cdot (\text{CH}_2)_{14} \cdot \text{COOH}$, stearic acid $\text{CH}_3 \cdot (\text{CH}_2)_{16} \cdot \text{COOH}$.

Dibasic fatty acids: oxalic acid $\begin{array}{c} \text{COOH} \\ | \\ \text{COOH} \end{array}$, malonic acid $\text{CH}_2 \begin{array}{c} \text{COOH} \\ \diagup \quad \diagdown \\ \text{COOH} \end{array}$, succinic acid $(\text{CH}_2)_2 \begin{array}{c} \text{COOH} \\ \diagup \quad \diagdown \\ \text{COOH} \end{array}$, azelaic acid $(\text{CH}_2)_7 \begin{array}{c} \text{COOH} \\ \diagup \quad \diagdown \\ \text{COOH} \end{array}$.

Hydroxy fatty acids: lactic acid $\text{CH}_3 \cdot \text{CH} \cdot \text{OH} \cdot \text{COOH}$, citric acid $\text{COOH} \cdot \text{CH}_2 \cdot \text{C}(\text{OH}) \cdot (\text{COOH}) \cdot \text{CH}_2 \cdot \text{COOH}$.

Results.

Results obtained are shown in Tables 110–125. From the data Figs. 56 is able to be drawn. K , t_1 and A/a calculated are given in Table 126.

Table 110. Influence of Na-formate.

Time of incubation	0.025 M			0.01 M			0.005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.865			0.865			0.865		
2	1.167	0.302	1.664	1.167	0.302	1.664	1.167	0.302	1.664
3	1.485	0.620	0.265	1.502	0.637	0.300	1.502	0.637	0.300
4	1.730	0.865	0.974	1.710	0.845	0.878	1.692	0.827	0.804
5	1.768	0.903	1.224	1.777	0.912	1.307	1.768	0.903	1.224
7	1.805	0.940	1.744	1.805	0.940	1.744	1.805	0.940	1.744
9	1.822	0.957		1.822	0.957		1.822	0.957	

Table 110. continued.

Time of incubation	0.0025 M			0.001 M			0.0005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.865			0.865			0.865		
2	1.167	0.302	1.664	1.167	0.302	1.664	1.167	0.302	1.664
3	1.502	0.637	0.300	1.485	0.620	0.265	1.485	0.620	0.265
4	1.710	0.845	0.878	1.710	0.845	0.878	1.730	0.865	0.974
5	1.768	0.903	1.224	1.777	0.912	1.307	1.768	0.903	1.224
7	1.805	0.940	1.744	1.805	0.940	1.744	1.805	0.940	1.744
9	1.822	0.957		1.822	0.957		1.822	0.957	

Table 110. continued.

Time of incubation	0.00025 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.865			0.865		
2	1.183	0.318	1.698	1.183	0.318	1.698
3	1.502	0.637	0.300	1.502	0.637	0.300
4	1.730	0.865	0.974	1.749	0.884	1.083
5	1.777	0.912	1.307	1.777	0.912	1.307
7	1.805	0.940	1.744	1.822	0.957	
9	1.822	0.957		1.822	0.957	

Table 111. Influence of Na-acetate.

Time of incubation	0.025 M			0.01 M			0.005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.677			0.677			0.677		
2	—	—	—	0.752	0.075	2.999	1.034	0.357	1.772
3	0.752	0.075	1.124	1.129	0.452	0.081	1.467	0.790	0.668
4	0.940	0.263	1.845	1.392	0.715	0.801	1.599	0.922	1.386
5	1.259	0.582	1.018	1.485	0.808	1.606	1.637	0.960	
7	1.315	0.638		1.505	0.828		1.637	0.960	
9	1.315	0.638		1.505	0.828		1.637	0.960	

Table 111. continued.

Time of incubation	0.0025 M			0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.677			0.677			0.677		
2	1.128	0.451	1.911	1.128	0.451	1.911	1.128	0.451	1.911
3	1.467	0.790	0.546	1.467	0.790	0.546	1.467	0.790	0.546
4	1.599	0.922	0.996	1.599	0.922	0.996	1.599	0.922	0.996
5	1.655	0.978	1.422	1.655	0.978	1.422	1.655	0.978	1.422
7	1.692	1.015		1.692	1.015		1.692	1.015	
9	1.692	1.015		1.692	1.015		1.692	1.015	

Table 112. Influence of Na-propionate.

Time of incubation	0.01 M			0.005 M			0.0025 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.752			0.752			0.752		
2	0.752	0		1.007	0.255		1.127	0.375	1.698
3	0.902	0.150	1.212	1.427	0.675	1.506	1.637	0.885	0.564
4	1.260	0.508	1.956	1.694	0.942	0.254	1.822	1.070	1.274
5	1.654	0.902	0.728	1.804	1.052	0.932	1.879	1.127	
7	1.823	1.071		1.804	1.052		1.879	1.127	
9	1.823	1.071		1.804	1.052		1.879	1.127	

Table 112. continued.

Time of incubation	0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.752			0.752		
2	1.147	0.395	1.690	1.204	0.452	1.730
3	1.637	0.885	0.446	1.767	1.015	0.558
4	1.879	1.127	1.178	1.917	1.165	0.950
5	1.954	1.202		2.012	1.260	1.544
7	1.954	1.202		2.048	1.296	
9	1.954	1.202		2.048	1.296	

Table 113. Influence of Na-butyrate.

Time of incubation	0.01 M			0.005 M			0.0025 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.752			0.752			0.752		
2	0.870	0.118	1.053	1.109	0.357	1.617	1.147	0.395	1.642
3	1.241	0.489	1.860	1.580	0.828	0.325	1.749	0.997	0.524
4	1.694	0.942	0.626	1.843	1.091	0.928	1.917	1.165	0.950
5	1.841	1.089	1.157	1.936	1.184	1.518	2.012	1.260	1.544
7	1.917	1.165		1.972	1.220		2.048	1.296	
9	1.917	1.165		1.972	1.220		2.048	1.296	

Table 113. continued.

Time of incubation	0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.752			0.752		
2	1.204	0.452	1.730	1.204	0.452	1.730
3	1.749	0.997	0.558	1.767	1.015	0.558
4	1.917	1.165	0.950	1.917	1.165	0.950
5	2.012	1.260	1.544	2.012	1.260	1.544
7	2.048	1.296		2.048	1.296	
9	2.048	1.296		2.048	1.296	

Table 114. Influence of Na-caproate.

Time of incubation	0.01 M			0.005 M			0.025 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.828			0.828			0.828		
2	0.828	0		0.865	0.037	2.668	0.941	0.113	1.136
3	0.865	0.037	2.775	0.979	0.151	1.344	1.185	0.357	1.787
4	0.997	0.169	1.540	1.241	0.413	1.992	1.580	0.752	0.603
5	1.316	0.488	0.459	1.542	0.714	0.775	1.693	0.865	1.062
7	1.486	0.658		1.662	0.834		1.768	0.940	
9	1.486	0.658		1.662	0.834		1.768	0.940	

Table 114. continued.

Time of incubation	0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.828			0.828		
2	1.110	0.282	1.586	1.129	0.301	1.626
3	1.373	0.545	0.065	1.430	0.602	0.164
4	1.693	0.865	0.761	1.693	0.865	0.761
5	1.768	0.940	1.098	1.768	0.940	1.098
7	1.843	1.015		1.843	1.015	
9	1.843	1.015		1.843	1.015	

Table 115. Influence of Na-nonylate.

Time of incubation	0.005 M			0.0025 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.771			0.771		
2	0.771	0		0.884	0.113	1.135
3	0.790	0.019	2.403	1.090	0.319	1.710
4	0.846	0.075	1.033	1.467	0.696	0.454
5	1.204	0.433	0.108	1.589	0.818	0.824
7	1.505	0.734	1.298	1.712	0.941	
9	1.542	0.771		1.712	0.941	

Table 115. continued.

Time of incubation	0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.771			0.771		
2	0.921	0.150	1.212	1.090	0.319	1.587
3	1.147	0.376	1.733	1.354	0.583	0.016
4	1.505	0.734	0.348	1.731	0.960	0.714
5	1.712	0.941	0.860	1.842	1.071	1.156
7	1.823	1.052	1.744	1.917	1.146	
9	1.842	1.071		1.917	1.146	

At 0.025 M and 0.01 M, the decomposition is very slightly.

Table 116. Influence of Na-laurate.

Time of incubation	0.01 M			0.005 M			0.0025 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.828			0.828			0.828		
2	0.828	0		0.828	0		0.828	0	
3	—	—	—	—	—	—	0.865	0.037	2.726
4	0.828	0		0.941	0.113	1.261	0.941	0.113	1.261
5	0.979	0.151	1.414	1.129	0.301	1.844	1.129	0.301	1.844
7	1.523	0.695	1.263	1.523	0.695	1.263	1.523	0.695	1.263
9	1.561	0.733		1.561	0.733		1.561	0.733	

Table 116. continued.

Time of incubation	0.00025 M			0.0001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.828			0.828			0.828		
2	1.129	0.301	1.626	1.129	0.301	1.626	1.129	0.301	1.626
3	1.430	0.602	0.164	1.430	0.602	0.164	1.430	0.602	0.164
4	1.693	0.865	0.761	1.693	0.865	0.761	1.693	0.865	0.761
5	1.768	0.940	1.094	1.768	0.940	1.094	1.768	0.940	1.094
7	1.843	1.015		1.843	1.015		1.843	1.015	
9	1.843	1.015		1.843	1.015		1.843	1.015	

Table 117. Influence of Na-myristate.

Time of incubation	0.0025 M			0.001 M			0.0005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.752			0.752			0.752		
2	0.827	0.075	2.977	0.940	0.188	1.432	1.053	0.301	1.625
3	1.034	0.282	1.685	1.297	0.545	0.207	1.335	0.583	0.131
4	1.391	0.639	0.452	1.523	0.771	0.835	1.579	0.827	0.644
5	1.560	0.808	1.152	1.617	0.865	1.659	1.674	0.922	0.996
7	1.617	0.865		1.636	0.884		1.750	0.998	1.769
9	1.617	0.865		1.636	0.884		1.767	1.015	

Table 117. continued.

Time of incubation	0.00025 M			0.0001 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.752			0.752		
2	1.053	0.301	1.625	1.053	0.301	1.625
3	1.335	0.583	0.131	1.335	0.583	0.131
4	1.560	0.808	0.592	1.560	0.808	0.592
5	1.674	0.922	0.996	1.674	0.922	0.996
7	1.750	0.998	1.769	1.767	1.015	
9	1.767	1.015		1.767	1.015	

Table 117. continued.

Time of incubation	0.00005 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.752			0.752		
2	1.072	0.320	1.664	1.072	0.320	1.664
3	1.372	0.620	0.196	1.372	0.620	0.196
4	1.542	0.790	0.546	1.542	0.790	0.546
5	1.674	0.922	0.996	1.674	0.922	0.996
7	1.750	0.998	1.769	1.750	0.998	1.769
9	1.767	1.015		1.767	1.015	

Table 118. Influence of Na-palmitate.

Time of incubation	0.0005 M			0.00025 M			0.0001 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.752			0.752			0.752		
2	0.977	0.225	1.510	0.977	0.225	1.510	1.072	0.320	1.664
3	1.203	0.451	1.981	1.222	0.470	0.017	1.372	0.620	0.196
4	1.447	0.695	0.487	1.447	0.695	0.487	1.542	0.790	0.546
5	1.560	0.808	0.851	1.560	0.808	0.851	1.674	0.922	0.996
7	1.655	0.903	1.677	1.655	0.903	1.677	1.750	0.998	1.769
9	1.674	0.922		1.674	0.922		1.767	1.015	

Table 118. continued.

Time of incubation	0.00005 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.752			0.752		
2	1.054	0.302	1.627	1.072	0.320	1.664
3	1.372	0.620	0.196	1.372	0.620	0.196
4	1.523	0.771	0.500	1.542	0.790	0.546
5	1.674	0.922	0.996	1.674	0.922	0.996
7	1.750	0.998	1.769	1.750	0.998	1.769
9	1.767	1.015		1.767	1.015	

Table 119. Influence of Na-stearate.

Time of incubation	0.0025 M			0.001 M			0.0005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.715			0.715			0.715		
2	—	—	—	0.771	0.056	2.842	0.921	0.206	1.496
3	0.771	0.056	2.842	1.053	0.338	1.807	1.185	0.470	0.076
4	1.033	0.318	1.765	1.353	0.638	0.449	1.410	0.695	0.612
5	1.353	0.638	0.449	1.543	0.828	1.350	1.523	0.808	1.152
7	1.543	0.828	1.350	1.580	0.865		1.580	0.865	
9	1.580	0.865		1.580	0.865		1.580	0.865	

Table 119. continued.

Time of incubation	0.0001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.715			0.715		
2	1.016	0.301	1.661	1.053	0.338	1.698
3	1.298	0.583	0.190	1.392	0.677	0.302
4	1.486	0.771	0.612	1.580	0.865	0.761
5	1.637	0.922	1.385	1.656	0.941	1.105
7	1.675	0.960		1.730	1.015	
9	1.675	0.960		1.730	1.015	

Table 120. Influence of Na-oxalate.

Time of incubation	0.05 M			0.025 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.734			0.734			0.734		
2	1.053	0.319	1.673	1.053	0.319	1.673	1.053	0.319	1.673
3	1.371	0.637	0.217	1.371	0.637	0.217	1.371	0.637	0.217
4	1.580	0.846	0.746	1.580	0.846	0.746	1.580	0.846	0.746
5	1.675	0.941	1.218	1.675	0.941	1.218	1.675	0.941	1.218
7	1.732	0.998		1.732	0.998		1.732	0.998	
9	1.732	0.998		1.732	0.998		1.732	0.998	

Table 121. Influence of Na-malonate.

Time of incubation	0.025 M			0.01 M			0.005 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.771			0.771			0.771		
2	0.959	0.188	1.398	0.959	0.188	1.398	1.034	0.265	1.534
3	1.241	0.470	0.000	1.241	0.470	0.000	1.285	0.514	1.997
4	1.617	0.846	0.954	1.617	0.846	0.954	1.636	0.865	0.710
5	1.693	0.922	1.710	1.693	0.922	1.710	1.749	0.978	1.142
7	1.711	0.940		1.711	0.940		1.805	1.034	
9	1.711	0.940		1.711	0.940		1.805	1.034	

Table 121. continued.

Time of incubation	0.0025 M			0.001 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.771			0.771			0.771		
2	1.090	0.319	1.587	1.090	0.319	1.587	1.090	0.319	1.587
3	1.373	0.602	0.045	1.354	0.583	0.016	1.354	0.583	0.016
4	1.749	0.978	0.765	1.731	0.960	0.714	1.731	0.960	0.714
5	1.861	1.090	1.290	1.842	1.071	1.156	1.842	1.071	1.156
7	1.917	1.146		1.917	1.146		1.917	1.146	
9	1.917	1.146		1.917	1.146		1.917	1.146	

Table 122. Influence of Na-succinate.

Time of incubation	0.05 M			0.025 M			0.01 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.715			0.715			0.715		
2	0.715	0		0.715	0		0.752	0.037	2.670
3	0.715	0		0.734	0.019	2.448	0.978	0.263	1.668
4	1.016	0.301	1.882	1.016	0.301	1.882	1.392	0.677	0.652
5	1.392	0.677	0.553	1.392	0.677	0.553	1.505	0.790	1.318
7	1.411	0.696		1.411	0.696		1.543	0.828	
9	1.411	0.696		1.411	0.696		1.543	0.828	

Table 122. continued.

Time of incubation	0.005 M			0.0025 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.715			0.715			0.715		
2	0.865	0.150	1.345	0.978	0.263	1.616	1.053	0.338	1.698
3	1.167	0.452	0.081	1.317	0.602	0.303	1.392	0.677	0.302
4	1.486	0.771	1.132	1.505	0.790	0.849	1.580	0.865	0.761
5	1.543	0.828		1.580	0.865	1.369	1.656	0.941	1.105
7	1.543	0.828		1.617	0.902		1.730	1.015	
9	1.543	0.828		1.617	0.902		1.730	1.015	

Table 123. Influence of Na-azelaate.

Time of incubation	0.05 M			0.025 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.734			0.734			0.734		
2	0.922	0.188	1.398	0.922	0.188	1.398	1.053	0.319	1.673
3	1.204	0.470	0.000	1.204	0.470	0.000	1.355	0.621	0.217
4	1.599	0.865	1.056	1.599	0.865	1.056	1.580	0.846	0.746
5	1.656	0.922	1.686	1.656	0.922	1.686	1.675	0.941	1.218
7	1.675	0.941		1.675	0.941		1.732	0.998	
9	1.675	0.941		1.675	0.941		1.732	0.998	

Table 124. Influence of Na-lactate.

Time of incubation	0.1 M			0.05 M		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.827			0.827		
2	0.846	0.019	2.448	0.977	0.150	1.322
3	0.883	0.056	2.942	1.241	0.414	1.963
4	1.147	0.320	1.931	1.504	0.677	0.557
5	1.391	0.564	0.631	1.654	0.827	1.348
7	1.523	0.696		1.692	0.865	
9	1.523	0.696		1.692	0.865	

Table 124. continued.

Time of incubation	0.02 M			Control		
	Determ.	y	$\log \frac{y}{A-y}$	Determ.	y	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.	
0	0.827			0.827		
2	1.090	0.263	1.492	1.090	0.263	
3	1.279	0.452	1.838	1.279	0.452	1.492
4	1.580	0.753	0.325	1.580	0.753	1.838
5	1.862	1.035	1.141	1.862	1.035	0.325
7	1.937	1.110		1.937	1.110	1.141
9	1.937	1.110		1.937	1.110	

At 0.2M, the decomposition does not take place.

Table 125. Influence of Na-citrate.

Time of incubation	0.1 M			0.05 M			0.02 M		
	Determ.	<i>y</i>	$\log \frac{y}{A-y}$	Determ.	<i>y</i>	$\log \frac{y}{A-y}$	Determ.	<i>y</i>	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.715	—	—	0.715	—	—	0.715	0	—
2	—	—	—	—	—	—	0.715	0	—
3	0.941	0.226	1.590	0.941	0.226	1.590	0.941	0.226	1.590
4	1.392	0.677	0.714	1.392	0.677	0.714	1.392	0.677	0.714
5	1.505	0.790	1.643	1.505	0.790	1.643	1.505	0.790	1.643
7	1.523	0.808		1.523	0.808		1.523	0.808	
9	1.523	0.808		1.523	0.808		1.523	0.808	

Table 125. continued.

Time of incubation	0.01 M			0.005 M			Control		
	Determ.	<i>y</i>	$\log \frac{y}{A-y}$	Determ.	<i>y</i>	$\log \frac{y}{A-y}$	Determ.	<i>y</i>	$\log \frac{y}{A-y}$
days	mg.	mg.		mg.	mg.		mg.	mg.	
0	0.715	—	—	0.715	—	—	0.715	—	—
2	0.771	0.056	2.873	0.884	0.169	1.398	1.053	0.338	1.698
3	0.941	0.226	1.590	1.091	0.376	1.904	1.392	0.677	0.302
4	1.392	0.677	0.714	1.430	0.715	0.737	1.580	0.865	0.761
5	1.505	0.790	1.643	1.523	0.808	1.328	1.656	0.941	1.105
7	1.523	0.808		1.561	0.846		1.730	1.015	
9	1.523	0.808		1.561	0.846		1.730	1.015	

Formic acid:—Higher concentration than 0.05 M does not permit the bacterial growth, so that the decomposition also does not occur. But the progress of the decomposition is independent of the concentration below 0.025 M, K , t_1 and A/a remaining the same as those of control. Namely, so far as bacteria are allowed to develop, the progress of the decomposition is not influenced by the presence of sodium formate.

Acetic acid:—There is no growth of bacteria at higher concentration than 0.05 M. At lower concentration than 0.025 M, both K and t_1 become smaller and A/a becomes larger with decreasing concentration. At the concentration lower than 0.0025 M, the no effect is recognized upon the decomposition.

Propionic acid:—The bacterial decomposition does not take place at higher concentration than 0.025 M. At the concentration lower than 0.01 M, A/a becomes larger and both K and t_1 become smaller

Table 126. Values of various constants.

Kind of salts	Mol. concentration	<i>a</i>	<i>A</i>	<i>A/a</i>	<i>K</i>	<i>t</i> ₁
		mg.	mg.			d.
Formate	0	4.046	0.957	0.235	0.550	2.55
	0.00025					
	0.0005					
	0.001					
	0.0025					
	0.005					
	0.01					
Acetate	0	4.841	1.015	0.210	0.540	2.30
	0.001					
	0.0025		0.960	0.198	0.775	2.30
	0.005					
	0.01					
Propionate	0	5.127	1.296	0.252	0.535	2.30
	0.001		1.202	0.235	0.780	2.45
	0.0025		1.127	0.220	0.780	2.45
	0.005		1.052	0.205	0.790	2.75
	0.01		1.071	0.208	0.790	4.05
Butyrate	0	5.127	1.296	0.252	0.535	2.30
	0.001					
	0.0025		1.220	0.238	0.640	2.65
	0.005					
Caproate	0	4.309	1.015	0.236	0.525	2.70
	0.001					
	0.0025		0.940	0.218	0.660	3.30
	0.005					
Nonylate	0	4.554	1.146	0.251	0.540	2.85
	0.001					
	0.0025		1.071	0.235	0.610	3.45
	0.005		0.941	0.206	0.610	3.45
	0.01		0.771	0.169	0.750	5.00
	0.025		0.433	0.095	—	—
Laurate	0	4.309	1.015	0.236	0.535	2.70
	0.0001					
	0.00025		0.733	0.170	0.610	5.10
	0.0025					
	0.005					
Myristate	0	4.573	1.015	0.222	0.475	2.85
	0.00005					
	0.0001		0.884	0.193	0.725	2.75
	0.00025					
	0.0005					
	0.001		0.865	0.189	0.725	3.45
	0.0025					

Table 126. continued.

Kind of salts	Mol. concentration	a	A	A/a	K	t_1
		mg.	mg.			d.
Palmitate	0	4.573	1.015	0.222	0.475	2.80
	0.00005					
	0.0001		0.922	0.201	0.475	3.15
	0.00025					
	0.0005					
Stearate	0	4.422	1.015	0.230	0.510	2.60
	0.0001		0.960	0.217	0.510	2.60
	0.0005		0.865	0.195	0.570	2.95
	0.001				0.790	3.40
	0.0025				0.840	4.35
Oxalate	0	4.177	0.998	0.238	0.545	2.65
	0.025					
	0.05					
Malonate	0	4.554	1.146	0.251	0.540	2.80
	0.001		1.034	0.227	0.540	2.80
	0.0025					
	0.005					
	0.01		0.940	0.206	0.775	2.90
Succinate	0	4.422	1.015	0.230	0.530	2.55
	0.0025		0.902	0.204	0.530	2.55
	0.005		0.828	0.187	0.825	2.80
	0.01				0.910	3.35
	0.025				1.555	4.20
	0.05		0.696	0.157		
Azelaate	0	4.403	0.998	0.226	0.540	2.60
	0.025		0.941	0.213	0.780	2.80
	0.05					
Lactate	0	5.176	1.110	0.215	0.510	3.05
	0.02		0.865	0.165	0.665	3.10
	0.05					
	0.1		0.696	0.135	0.790	4.15
Citrate	0	4.422	1.015	0.230	0.540	2.55
	0.005		0.846	0.191	0.700	2.90
	0.01		0.803	0.182	0.950	3.30
	0.02					
	0.05					
	0.1					

with decreasing concentration. This effect is recognized even at the concentration of 0.001 M.

Butyric acid:—The bacterial decomposition does not take place, if the concentration is higher than 0.025 M. At the concentration lower than 0.01 M, A/a becomes larger and both K and t_1 become smaller with decreasing concentration. But the effect is not inflicted on the decomposition at lower concentration than 0.0025 M.

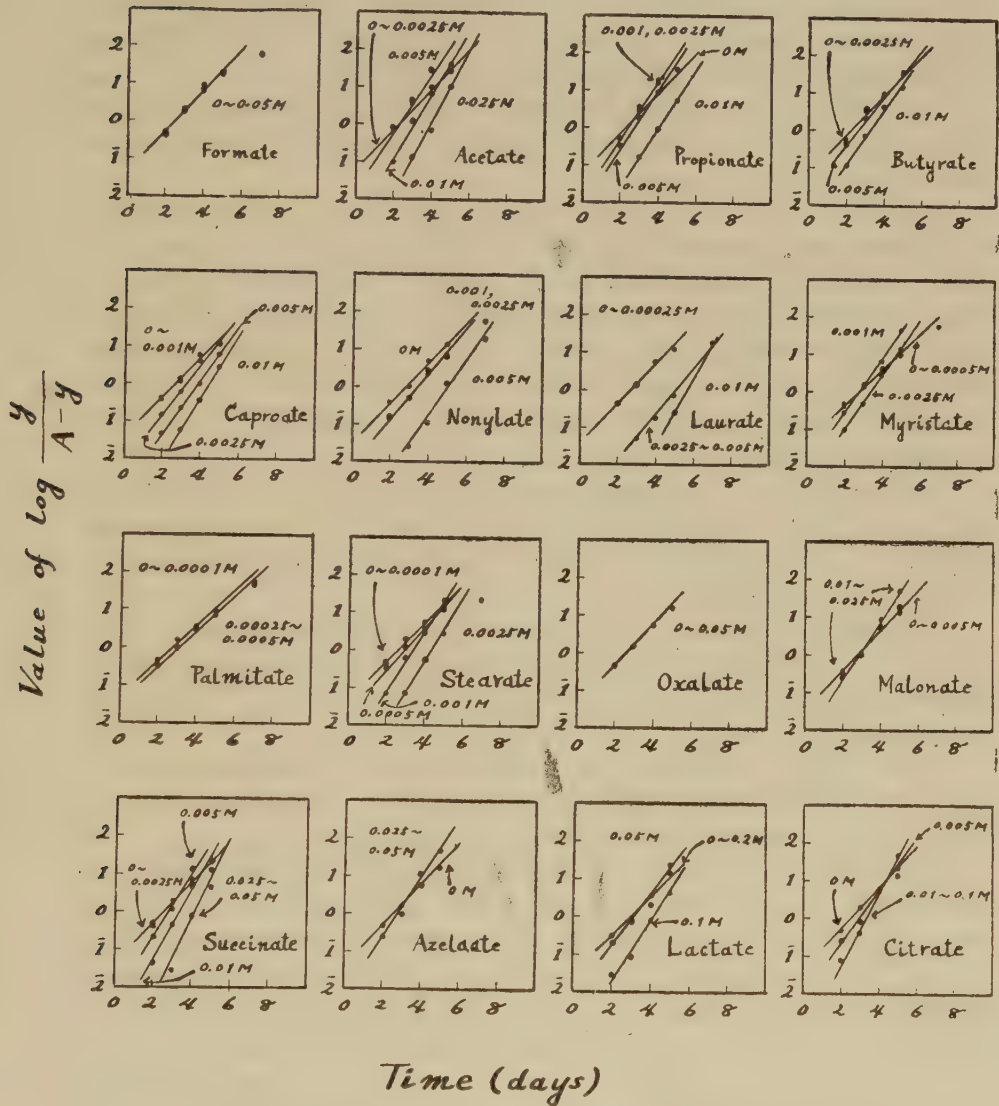


Fig. 56. Influence of Na-salts of various organic acids on the bacterial decomposition (relation between $\log \frac{y}{A-y}$ and time).

Caproic acid:—There is no growth of bacteria at higher concentration than 0.025 M, so that the decomposition does not take place. At lower concentration than 0.01 M, A/a becomes larger and both K and t_1 become smaller with decreasing concentration. This effect is not recognized at lower concentration than 0.001 M.

Nonylic acid:—The effect is shown even at the concentration of 0.001 M, and A/a becomes smaller and both K and t_1 become larger with increasing concentration.

Lauric acid:—There is no effect at lower concentration than 0.00025 M. At higher concentration than 0.0025 M, A/a is smaller and both K and t_1 are larger than those of the control, but they are almost unchanged with varying concentrations. From this reason, it seems probable that when more quantity of this salt is added, its total amount is not dissolved into the medium but a part of it remains as precipitate, that is the solution is no more concentrated, but only the amount of the precipitate increases.

Myristic acid:—The effect is not recognized at lower concentration than 0.0005 M. At higher concentration than 0.001 M, A/a becomes smaller and both K and t_1 become larger. But it seems to have a tendency as lauric acid.

Palmitic acid:—The decomposition is not influenced at lower concentration than 0.0001 M. At higher concentration than that, A/a is smaller, and both K and t_1 are larger, but they are little influenced by varying concentrations.

Stearic acid:—The effect is inflicted on the decomposition even at the concentration of 0.0001 M. A/a becomes smaller and both K and t_1 become larger with increasing concentration.

Oxalic acid:—Higher concentration than 0.05 M was unable to be obtained, for its sodium salt is slightly dissolved into water. At lower concentration than that, there is no effect on the decomposition.

Malonic acid:—There is no effect at lower concentration than 0.0025 M. At higher concentration than 0.005 M, the effect is slightly recognized, and A/a is smaller and both K and t_1 are larger.

Succinic acid:— A/a becomes smaller and both K and t_1 become larger with increasing concentration. The decomposition takes place even at the concentration of 0.05 M.

Azelaic acid:—The decomposition is comparatively little affected. The progress of the decomposition is not very varied even at the concentration of 0.05 M.

Lactic acid:—There is no effect at lower concentration than

0.02 M. At higher concentration than that, A/a becomes smaller and both K and t_1 become larger with increasing concentration. But there is no growth of bacteria at higher concentration than 0.2 M, so that the decomposition does not take place.

Citric acid:— A/a becomes smaller and both K and t_1 become larger with increasing concentration, but the difference is very small. The decomposition takes place even at the concentration of 0.1 M.

Summary.

The above mentioned results may be summarized as follows:

(1) Na-salts of the organic acids have variable effects on the bacterial decomposition. They increase both the values of K and t_1 , but decrease the value of A/a . In other words, they retard the time requiring for the beginning of rapid decomposition (i.e. inductive period), but after this period they increase the speed of the decomposition, though limit the final rate of the decomposition.

(2) With monobasic fatty acids, the greater the molecular weight, the more the effects are observed at the lower concentration. But, as the degree of dissolution has certain limit for those acids, of which molecular weight is greater than lauric acid, the effects are indifferent to concentrations of such acids above this limit.

(3) So far as the present study is concerned, the effects in question are the greatest for monobasic fatty acids, next for dibasic fatty acids, and the least for hydroxyfatty acids.

General Summary and Conclusions.

In the present paper, the decomposing bacteria for the meat of various aquatic animals and the progress of the decomposition caused by them, were studied from various standpoints.

1. The spoilage of meat of aquatic animals is presumed to be caused by water bacteria belonging to *Pseudomonas*, *Achromobacter* and *Flavobacterium*.

2. When the bacterial growth nearly reaches the maximum, the decomposition of the muscle is suddenly progressed.

3. After the bacteria were introduced, the time required for them to reach the maximum growth is nearly the same irrespective of the number of the initial inoculated cells. At the maximum growth, the number of cells is approximately constant. The genera-

tion time of bacteria depends on the amount of the inoculum and becomes longer with larger amount of the inoculum.

4. The progress of the bacterial decomposition is approximately independent of the number of bacterial cells introduced in the inoculum.

5. The progress of the bacterial decomposition of muscles, meat extracts and muscle proteins of aquatic animals can be defined by the equation

$$\log \frac{y}{A-y} = K(t - t_1),$$

between y , the metabolic product and t , the time with three constants, K , t_1 and A . Therefore, if these three constants are examined, the progress of the bacterial decomposition becomes clear. The constant K , representing the speed of the decomposition, depends on the kind of the materials which are decomposed; that is, in the case of the same material it is nearly the same irrespective of the concentration of substrate under the same condition. The constant t_1 , representing the period in which the reaction is half completed, i. e.

$y = \frac{A}{2}$, is dependent of the original concentration in the same kind

of the substrate, and becomes larger with increasing concentration. The constant A , representing the total amount of the metabolic product, i. e. $y = \text{maximum}$, is influenced by increasing or decreasing concentration of the original substrate, because it is a fractional part of a , representing the original concentration of the substrate. A/a is independent of the original concentration of the substrate of the same kind.

6. The speed of the bacterial decomposition of meat extracts, if compared by the value of K , is the greatest with red meat fishes (such as bonito, chub mackerel, etc.), the smallest with white meat fishes (such as serranid, sea bream, halibut, etc.), and intermediate with carangoid. With crustacea (such as spiny lobster) it is greater than with fishes. The speed of the decomposition of muscle proteins is greater with crustacea and the dark muscle of fish than with the other fishes, for which it is nearly the same irrespective of the kinds.

7. From these facts, it is presumed that, in the case of the meat of aquatic animals, the speed of the bacterial decomposition of crustacea is greater than that of fishes, because the circumstances are so for both extractive matter and muscle protein. The speed of bacterial decomposition of fishes is decided by that of the extractive matter,

because that of the muscle proteins of any fishes is almost the same.

8. With the same kind of animal, the speed of the bacterial decomposition of the meat extract decreases with increasing age of that animal up to a certain definite age, and it becomes nearly the same irrespective of age above that age. Therefore, it is presumed that the muscle of younger animal is easily decomposed by bacteria than that of older, but it is nearly indifferent of age above a certain definite age.

9. The value of A/a , representing the final rate of the bacterial decomposition, varies approximately with the same tendency as the value of K , that is, when K is large, A/a is also large. But the dark muscle is an exception, with which although K is large, A/a is small.

10. In the case of the mixture of two kinds of meat extracts, any one of three constants K , t_1 and A/a lies between those of the two components of the mixture, and resembles nearly that of the component much contained in the mixture.

11. The difference of the type of the bacterial decomposition of meat extracts depends on the chemical composition of the extractive matter which varies with kind of animals, and the unknown substance seems to be not present in the meat extracts, which accelerates or inhibits the bacterial decomposition.

12. The growth of bacteria in the solution of meat extract or in the muscle suspension is independent of the kind of animals, if the original concentration of the substrate is not very dilute. It is understood that the bacterial growth is similar, although the type of the bacterial decomposition varies with the kind of animals.

13. In the case of the bacterial decomposition of meat of aquatic animals, the increase of autolytic products accumulated in the meat has little influence upon the values of K and A/a , if the amount of autolytic products is not very much. However, the value of t_1 becomes smaller with increasing of the amount of the autolytic products. In other words, the time required for the beginning of the rapid increase of the decomposition (i.e. length of the inductive period), becomes shorter with length of time autolysis has gone on, but after this time (i.e. at the end of the inductive period) the speed of the decomposition is almost the same irrespective of the degree of autolysis.

14. In the case of combined action of both bacteria and autolytic enzymes, although both K and t_1 are approximately the same as

those in the case of bacterial action only; A/a in the former case is larger than that in the latter, i.e. during the same length of time after inoculation of bacteria, the decomposition in the former is larger than in the latter. Therefore, it is presumed that raw meat is decomposed more than heated meat by bacteria.

15. The growth of bacteria is not almost influenced by both the amount of autolytic products and autolytic enzymes.

16. The effect of autolysis on the bacterial decomposition of meat of aquatic animals is concluded as follows:

The time required to begin the increase of the bacterial decomposition rapidly, becomes shorter by the increase of autolytic products, and the degree of the decomposition becomes larger by autolytic enzymes, but the speed of the decomposition is not influenced.

17. The bacterial decomposition is influenced by the environmental temperature. The effect of temperatures on the value of K is satisfied by ARRHENIUS' equation and temperature coefficient Q_{10} is about 2.5 between optimum temperature and about 15°C , and about 8-10 below about 15°C . The value of t_1 becomes larger with lowering of temperature, but A/a is independent of temperature. Therefore, it is evidently understood that the degree of the bacterial decomposition during the same time decreases with lowering of temperature and that the degree of the decrease is rather remarkable in lower temperature than about 15°C .

18. Injurious effect is given on the bacterial growth by the lowering of temperature. But the degree of the effect upon the bacterial growth is smaller than that upon the bacterial action (i.e. decomposing action). It may be presumed that the decomposition may be little permitted to develop at low temperature, in which bacteria may be allowed to develop.

19. When the surface tension of the substrate (which is the solution of meat extract) was depressed by addition of various amounts of Na-recinoleate (0.01-0.0005%) as the depressant, the progress of its bacterial decomposition is influenced as follows:

The value of K increases with depression in the initial surface tension of the substrate, but both A/a and t_1 are approximately the same without regard to the surface tension. Thus, it is understood that, in the case of the depression in the initial surface tension, the degree of the bacterial decomposition is smaller in the early stage, but is rapidly increases along the progress of the decomposition and

the decomposition is completed rapidly (i.e. the equilibrium is reached).

20. The surface tension changes with the decomposition of the substrate, but ceases to change when the progress of the decomposition reaches equilibrium, and at this time it becomes almost the same irrespective of the different initial surface tension.

21. Both the bacterial growth and the bacterial decomposition are affected by halogen ions and both do not take place above a certain definite concentration of them. The order of their inhibitory action is $F' > I' > Br' > Cl'$. The degree of the effect on the growth is different from that on the decomposition, and the latter seems to be influenced in lower concentration than the former. The decomposition seems to be slightly stimulated in a certain definite concentration of halogen ions excepting F' .

22. When the sodium salts of alkali metals or alkali-earth metals is present in the medium in concentration above a certain limit, both the bacterial growth and the bacterial decomposition do not take place. This concentration generally is lower in alkali-earth metals than in alkali metals. Below this concentration, the bacterial decomposition seems to be slightly increased at certain concentration of alkali metals, although it may be little influenced by alkali metals. But by alkali-earth metals it seems to be considerably influenced, with increasing in some cases, or decreasing in other cases. In the case of the same salt, the decomposition seems to be influenced at lower concentration than the growth of bacteria.

23. Various metallic salts inflict the remarkable effect on the progress of the bacterial decomposition of fish muscle. The value of A (representing the final rate of the decomposition) is either increased or decreased according to the kind and the concentration of metallic salts employed, but generally the value of K is not increased by any one of the metallic salts employed but is unchanged or decreased. The effect of metallic salts is divided into three groups according to their kinds, as follows:

(1) $AlCl_3$, $MnCl_2$ and $FeCl_3$ increase the value of A with increasing concentration of them up to a certain definite and comparatively high concentration but retard it markedly in higher concentration than that, whereas the value of K is almost unchanged, exclusive of very high concentration. (2) In the cases of $NiCl_2$, $CoCl_2$, $SnCl_2$, $AgNO_3$, $PtCl_4 \cdot 2HCl$ and $AuCl_3 \cdot HCl$, both A and K are not increased but decreased even at comparatively low concentration. (3) These do not belong to any one of the former two groups. $ZnSO_4$

increases the value of A within the range of certain concentration, but decreases the value of K even at low concentration. Although HgCl_2 resembles (1)-group, the effect is very slight and is shown at very low concentration.

24. The bacterial decomposition varies with hydrogen ion concentration of the medium. Its optimum hydrogen ion concentration is at about $\text{pH} = 6.0-7.0$. The degree of the effect on the growth of bacteria is smaller than that on the decomposition. The optimum hydrogen ion concentration for the growth is at about $\text{pH} = 6.0-8.0$.

25. When the fermentable carbohydrates are present in the substrate, the bacterial decomposition is remarkably repressed. The cause of this effect seems to be the complex action which is probably due not only to the hydrogen ion of acids produced, but also to the molecules or the anions of various acids and other fermentation products. But, the effect on the growth rate of bacteria is almost negligibly small, although the final amount of living cells is slightly influenced.

26. Sodium salts of various organic acids have effects on the bacterial decomposition. They increase both the values of K and t_1 , but decrease the value of A/a , that is, when the organic acid is present in the substrate, the time requiring to the beginning of rapidly decomposition (i.e. inductive period) becomes longer, and after this time the decomposition rapidly progresses and is soon completed, but the final rate of the decomposition becomes smaller. With mono-basic fatty acids, the greater the molecular weight, the more the effects are observed at the lower concentration. But, as the degree of dissolution has certain limit for those acids, of which molecular weight is greater than lauric acid, the effects are indifferent to concentrations of such acids over this limit. The effects in question are the greatest for mono-basic fatty acids, next for di-basic fatty acids, and the least for hydroxyfatty acids.

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